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(54) Title: HUMAN INTERLEUKIN-1 RECEPTOR ACCESSORY PROTEIN (57) Abstract This invention is directed to polynucleotides encoding human IL-1 receptor accessory protein, isolated IL-1 receptor accessory protein, and antibodies to IL-1 receptor accessory protein. This protein is particularly useful to prevent inflammation due to the action of IL-1.			

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Human Interleukin-1 Receptor accessory protein

The present invention relates generally to cytokine receptors, and more specifically to accessory proteins of interleukin 1 receptors.

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Interleukin 1 (IL-1) is a polypeptide hormone that acts on a variety of cell types and has multiple biological properties (Dinarello, Blood 77: 1627, 1991). IL-1 is a major mediator of inflammatory and immune responses. Therefore, regulation of IL-1 activity provides a
10 means of controlling and modulating these responses.

Two species of IL-1 have been characterized, interleukin 1 α (IL-1 α) and interleukin 1 β (IL-1 β), both of which are referred to herein as IL-1. The biological activities produced by IL-1 are
15 mediated by binding to specific plasma membrane receptors, termed the Type I and Type II IL-1 receptors. The IL-1 receptors (IL-1R's) are transmembrane proteins with extracellular domains of about 300 amino acids, and are members of the immunoglobulin superfamily of molecules (Sims et al., Science 241: 585, 1988; Sims et al., Proc. Natl.
20 Acad. Sci. USA 86: 8946, 1989; McMahan et al., EMBO J. 10: 2821, 1991). Both IL-1 species bind to each of these receptors and compete completely with each other for binding.

It has been assumed that the Type I IL-1R encodes the entire
25 functional IL-1 receptor. Experiments with the cloned Type I IL-1R indicated that when this receptor protein was transfected and expressed in Chinese hamster ovary cells, it was sufficient to bind IL-1 and to transduce the IL-1 signal (Curtis et al., Proc. Natl. Acad. Sci. USA 86: 3045, 1989). The presence of an accessory protein
30 endogenous to the hamster cells was not determined in these studies. It had been suggested that the Type II IL-1R represented an accessory chain of the IL-1R (Solari, Cytokine 2: 21, 1990). However, more recent studies have shown that the Type II IL-1R is unlikely to function as a signal-transducing accessory protein, and that it acts

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instead as a decoy receptor to bind excess IL-1 and regulate its activity (Colotta et al., Science 261: 472, 1993).

Since IL-1 binding to the IL-1 receptor mediates the biological effects of IL-1, an understanding of the mechanism of receptor binding and activation is important for regulating IL-1's activities. Affinity crosslinking and binding studies with labelled IL-1 have shown that the IL-1 receptor exists as a complex of multiple proteins that can bind IL-1 with different affinities (Lowenthal and MacDonald, J. Exp. Med. 164: 1060, 1986; Bensiman et al., J. Immunol. 143:1168, 1989; McMahan et al., EMBO J. 10:2821, 1991). A murine monoclonal (mAb) 4C5 has been described that recognizes a 90 kDa protein on murine cells that is associated with IL-1R and is required for signal transduction and biological activity (Powers et al., AAI meeting, Denver, CO, May 21-25, 1993). It was not known if an equivalent protein existed on human cells, or what biological function, if any, was associated with such a protein.

Prior to the present invention, efforts to identify a human IL-1R accessory protein or to clone and express genes encoding this protein have been significantly impeded by lack of purified protein, lack of an antibody that recognizes this protein, and inability to identify cells that express large amounts of this protein and its mRNA. Even the murine accessory protein had not been obtained in sufficient amounts to use in efforts to identify the corresponding human accessory protein. Murine cell lines known to express the accessory protein did so only in amounts (~1000 molecules/cell) too low to purify sufficient protein for obtaining unambiguous amino acid sequence information. There was no mAb known to recognize a human homologue of the 4C5 target protein (the murine accessory protein). In addition, binding to IL-1 was not known to be an effective screen for identifying a human accessory protein, since it is known that many accessory proteins do not bind ligand or bind with very low affinity (Hibi et al., Cell 63: 1149, 1990; Takeshita et al., Science 257: 379, 1992).

This invention makes available for the first time purified human IL-1 receptor accessory protein which can be used to regulate the effects of IL-1. The addition of soluble accessory protein inhibits the

effect of IL-1 on the cells. Hence, an aspect of the invention is the treatment of pathological conditions caused by excess activity of cells responding to IL-1 by adding an amount of soluble human IL-1R accessory protein (IL-1R AcP) sufficient to inhibit activation of cells
5 by IL-1. This methodology can also be modified, and the soluble accessory protein can be used as a screening agent for pharmaceuticals.

Briefly, a pharmaceutical which works as an IL-1 antagonist can
10 do so by blocking the interaction of IL-1 with the IL-1R AcP. The presence of IL-1R AcP in a cell membrane is necessary to permit IL-1 to interact effectively with the IL-1 receptor complex (by effective interaction is meant binding to the receptor complex so as to initiate a biological response). The IL-1 receptor complex includes the Type I or
15 Type II IL-1 receptor in association with the IL-1R AcP (additional proteins may also be part of the complex). Adding soluble IL-1R AcP inhibits this interaction by allowing IL-1 or the IL-1 receptor to interact with the soluble protein instead of IL-1R AcP on the cell surface, thus reducing the biological response caused by IL-1.
20 Antibodies to the IL-1R AcP of this invention similarly inhibit the biological response of cells to IL-1. By binding to the IL-1R AcP, antibodies prevent IL-1 from interacting effectively with the IL-1 receptor. By blocking IL-1R AcP, these antibodies inhibit the binding of IL-1 to the IL-1 receptor complex, which depends on interaction
25 with IL-1R AcP. IL-1R AcP will inhibit IL-1 interaction with the IL-1 receptor, thus preventing activation of IL-1 responsive cells and decreasing the inflammatory response. One may also use the purified IL-1R AcP to screen a potential pharmaceutical. If the pharmaceutical blocks IL-1 binding to the IL-1R AcP, it will be an effective IL-1
30 antagonist.

The present invention provides polynucleotides which encode IL-1 receptor accessory proteins or active fragments thereof, preferably, the polynucleotides are selected from a group consisting
35 of (a) polynucleotides, preferably cDNA clones, having essentially a nucleotide sequence derived from the coding region of a native IL-1R AcP gene, such as shown in Figure 15 [SEQ ID NO. 1]; (b) polynucleotides capable of hybridizing to the cDNA clones of (a) under

moderately stringent conditions and which encode IL-1R AcP or fragments thereof; and (c) polynucleotides which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode IL-1R AcP molecules or fragments thereof.

5 Particularly preferred compounds are the polynucleotides which encode human IL-1 receptor accessory proteins, e. g. the polynucleotides encoding the amino acid sequence [SEQ ID NO:3] or an active fragment thereof, especially a polynucleotide having the sequence [SEQ ID NO:1]. Especially preferred compounds encode
10 soluble IL-1 receptor accessory proteins, e. g. human soluble IL-1 receptor accessory proteins having for example the amino acid sequence [SEQ ID NO:9]. The polynucleotide [SEQ ID NO:7] codes for a human soluble IL-1 receptor accessory protein. Also part of this invention are the antisense polynucleotides of the above compounds.

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The present invention also provides vectors and suitable host cells, preferably expression vectors comprising the DNA sequences defined above, recombinant IL-1R AcP produced using the expression vectors, and a method for producing the recombinant accessory
20 protein molecules utilizing the expression vectors.

The present invention makes available IL-1 receptor accessory proteins and active fragments thereof, encoded by polynucleotides as defined above. Preferred compounds are human IL-1 receptor
25 accessory proteins, preferably a protein having the amino acid sequence [SEQ ID NO:3]. Especially preferred are soluble human IL-1 receptor accessory proteins, e. g. having the amino acid sequence [SEQ ID NO:9]. Also part of this invention are IL-1R AcP proteins carrying one or more side groups which have been modified.

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The present invention also provides antibodies to IL-1R AcP. These antibodies bind specifically to the human IL-1 receptor accessory protein and prevent activation of the IL-1 receptor complex by IL-1. The preferred antibodies have a binding affinity to the IL-1
35 receptor accessory complex of from about K_D 0.1 nM to about K_D 10 nM and are for example monoclonal antibodies or derivatives thereof.

Also part of this invention are pharmaceutical compositions which comprise an antisense polynucleotide, a IL-1 receptor accessory protein or an antibody as described above. These pharmaceutical compositions may include one or more other cytokine antagonists.

The invention also provides a process for the preparation of an IL-1 receptor accessory protein comprising the steps of (a) expressing a polypeptide encoded by an above mentioned polynucleotide in a suitable host, (b) isolating said IL-1 receptor accessory protein, and (c) if desired, converting it in an analogue wherein one or more side groups are modified. Moreover, the invention includes a process for the preparation of an IL-1 receptor accessory protein antibody comprising the steps of (a) preparation of a hybridoma cell line producing a monoclonal antibody which specifically binds to the IL-1 receptor accessory protein and (b) production and isolation of the monoclonal antibody. Corresponding polyclonal antibodies may be produced using known methods.

The above mentioned compounds are useful as therapeutically active substances, e. g. for use in the treatment of inflammatory or immune responses and/or for regulating and preventing inflammatory or immunological activities of Interleukin-1. Especially, these compounds are useful in the treatment of acute or chronic diseases, preferably rheumatoid arthritis, inflammatory bowel disease, septic shock, transplant rejection, psoriasis, asthma and Type I diabetes or, in the treatment of cancer, preferably acute and chronic myelogenous leukemia.

As used herein, IL-1 includes both IL-1 α and IL-1 β , and IL-1 receptor includes Type I and Type II IL-1 receptors, unless otherwise specifically indicated.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. Equilibrium Binding of [125 I]-4C5 to Murine EL-4 Cells at Room Temperature. EL-4 cells (1.5×10^6 cells) were

incubated for 2 hrs at room temperature with increasing concentrations of [125 I]-4C5 in the absence (o) or presence (∇) of 100 nM unlabeled 4C5. Total (o) and non-specific (∇) cell bound radioactivity were determined as described in Example 1. Specific binding of [125 I]-4C5 (•) was calculated by subtracting non-specific binding from total binding. 1A. Binding of EL-4 cells incubated with [125 I]-4C5. 1B. Analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213, 1985) with a single-site model.

Figure 2. Equilibrium binding of [125 I]-4C5 to Murine-70Z/3 Cells. 70Z/3 cells (1.5×10^6) were incubated for 2 hrs at room temperature with increasing concentrations of [125 I]-4C5 in the absence (o) or presence (∇) of 100 nM unlabeled 4C5. Total (o) and non-specific (∇) cell bound radioactivity were determined as described in Example 1. Specific binding of [125 I]-4C5 (•) was calculated by subtracting non-specific binding from total binding. 2A. Binding of 70Z/3 cells incubated with [125 I]-4C5. 2B. Analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213, 1985) with a single-site model.

Figure 3. Inhibition of Human [125 I]-IL-1 Binding to IL-1 Receptor on 70Z/3 Cells by Monoclonal Antibodies 4C5, 4E2 and 35F5. Inhibition assays were performed as described in Example 1. The data are expressed as the percent inhibition of [125 I]-IL-1 binding in the presence of the indicated concentrations of antibody when compared to the specific binding in the absence of antibody. Proteins are human IL-1 α (H-alpha) and human IL-1 β (H-beta).

Figure 4. Inhibition of Human [125 I]-IL-1 Binding to IL-1 Receptor on EL-4 Cells by Monoclonal Antibodies 4C5, 4E2 and 35F5. Inhibition assays were performed as described in Example 1. The data are expressed as the percent inhibition of [125 I]-IL-1 binding in the presence of the indicated concentrations of antibody when

compared to the specific binding in the absence of antibody. Proteins are human IL-1 α (H-alpha) and human IL-1 β (H-beta).

Figure 5. Isolation of Two Proteins of 90 and 50 kDa from a Solubilized Extract of EL-4 Cells by 4C5 Affinity Chromatography. Proteins were partially purified from a detergent extract of EL-4 cells by lentil lectin affinity chromatography followed by affinity chromatography on a matrix containing either an anti-Type I IL-1R antibody (7E6), murine IL-1 α (Ma) or anti-accessory protein antibody (4C5) as described in Example 1. Proteins in the detergent extract of EL-4 cells were also directly purified on a 4C5 affinity matrix (4C5). The proteins eluted from the columns were separated by SDS-PAGE, transferred to nitrocellulose and probed with [125 I]-4C5. The molecular sizes indicated in the margins were estimated from molecular weight standards (Amersham Prestained Standards) run in parallel lanes. Exposure time was 1 day.

Figure 6. Inhibition of IL-1 Induced Splenic B Cell Proliferation by Monoclonal Antibodies 4C5, 4E2 and 35F5. Inhibition assays were performed as described in Example 1. The data are expressed as the incorporation of 3 H-thymidine (CPM) by B cells in the presence of the indicated concentrations of antibody when compared to the incorporation in the absence of antibody. Proteins are: 6A. human IL-1 α (IL-1 α) and 6B. human IL-1 β (IL-1 β).

Figure 7. Inhibition of IL-1 Induced Proliferation of D10.G4.1 Helper T-cells by Monoclonal Antibodies 4C5 and 35F5 and Human IL-1ra. Inhibition assays were performed as described in Example 1. The data are expressed as the incorporation of 3 H-thymidine (CPM) by D10 cells in the presence of the indicated concentrations of antibody and IL-1ra when compared to the incorporation in the absence of antibody or IL-1ra. Proteins are: 7A. human IL-1 α , 7B. human IL-1 β .

Figure 8. Inhibition of IL-1 Induced Kappa Light Chain Expression by 70Z/3 Cells: Effect of Monoclonal Antibodies 4C5, 4E2 and 35F5. The induction of kappa light chain expression and inhibition with the antibodies was as described in Example 1. The

data are expressed as the percent of cells expressing kappa light chain in the presence of the indicated concentrations of antibody when compared to the percent of cells in the absence of antibody. Proteins are human IL-1 α (IL-1 α) and human IL-1 β (IL-1 β).

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Figure 9. Inhibition of IL-1 Induced Serum IL-6 in C57BL/6 Mice by Monoclonal Antibodies 4C5 and 35F5. Mice were pretreated with the monoclonal antibody at 4 hrs and 10 mins prior to subcutaneous injection of human IL-1 α (alpha) or human IL-1 β (beta) (0.03 μ g). Two hours after the IL-1 administration, the serum IL-6 concentration was determined as described in Example 1. Mab X-7B2 is a control antibody.

Figure 10. Nucleotide Sequence and Deduced Amino Acid Sequence of Murine IL-1R AcP. 10A. The nucleotide sequence of the opening reading frame of murine IL-1R AcP cDNA clone E2-K is shown. The top strand is the coding sequence [SEQ ID NO:4]. 10B. The amino acid sequence of murine IL-1R AcP as deduced from the coding sequence shown in Figure 10A is shown [SEQ ID NO:6]. The signal peptide cleavage site is predicted to occur after Ala -1, resulting in a 550 amino acid mature protein that extends from Ser 1 to Val 550. The cleavage site has been confirmed by NH₂-terminal sequence analysis of purified natural muIL-1R AcP (Example 10). The predicted transmembrane domain extends from Leu 340 through Leu 363.

Figure 11. Immunoprecipitation of Recombinant MuIL-1R AcP from Transfected COS cells with mAbs 4C5 and 2E6. COS cells were transfected by electroporation with either pEF-BOS/muIL-1R AcP or pEF-BOS alone (mock). Transfected cells were metabolically labelled with [³⁵S]Met as described (Example 8). Labelled transfectants were solubilized with RIPA buffer and immunoprecipitated with either mAb 4C5 or 2E6 (see Table 2) as described (Example 8). Both mAbs immunoprecipitated labelled protein from COS cells transfected with pEF-BOS/muIL-1R AcP which migrated as a broad band between 70-90 kDa. No labelled protein was detected in this size range from mock transfected COS cells. A higher molecular weight species (>200 kDa) is present in both mock and muIL-1R AcP transfected COS cells.

Figure 12. Equilibrium Binding of [125 I]-Labeled 4C5 and IL-1 to Murine Recombinant IL-1R AcP Expressed in COS-7 Cells. Cells ($4-8 \times 10^4$) transfected with an IL-1R AcP expression plasmid [COS(AcP)] or control plasmid [COS(PEF-BOS)] were incubated for 3 hrs at 4°C with increasing concentrations of [125 I]-4C5 or [125 I]-IL-1 α in the absence (Total) or presence (Non-Specific) of 100 nM unlabeled 4C5 or 50 nM unlabeled IL-1 α . Total (Total) and non-specific (Non-Specific) cell bound radioactivity were determined as described in Example 1. Specific binding of [125 I]-4C5 (Specific) and [125 I]-IL-1 α (Specific) were calculated by subtracting non-specific binding from total binding. The binding of [125 I]-IL-1 α to COS cells transfected with the control plasmid [COS(PEF-BOS)] showed that Cos-7 cells naturally express approximately 600 high affinity binding sites for IL-1 α . The right hand panel shows analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213, 1985) with a single-site model. 12A. Binding of COS(AcP) cells incubated with [125 I]-4C5 12B. Scatchard plot of 12A data. 12C. Binding of COS(AcP) cells incubated with [125 I]-IL-1 α 12D. Scatchard plot of 12C data. 12E. Binding of [COS(PEF-BOS)] cells incubated with [125 I]-IL-1 α . 12F. Scatchard plot of 12E data.

Figure 13. Equilibrium Binding of [125 I]-Labeled 35F5 and IL-1 to Murine Recombinant Type I IL-1R Expressed in COS-7 Cells. Cells ($4-8 \times 10^4$) transfected with an Type I IL-1R expression plasmid [COS(Mu-IL-1R)] were incubated for 3 hrs at 4°C with increasing concentrations of [125 I]-35F5 or [125 I]-IL-1 α and [125 I]-IL-1 β in the absence (Total) or presence (Non-Specific) of 100 nM unlabeled 35F5 or 50 nM unlabeled IL-1. Total (Total) and non-specific (Non-Specific) cell bound radioactivity were determined as described in Example 1. Specific binding of [125 I]-35F5 (Specific) and [125 I]-IL-1 α or IL-1 β (Specific) were calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213,

1985) with a single-site model. 13A. Binding of [COS(Mu-IL-1R)] cells incubated with [¹²⁵I]-35F5. 13B. Scatchard plot of 13A data. 13C. Binding of [COS(Mu-IL-1R) cells incubated with [¹²⁵I]-IL-1 β 13D. Scatchard plot of 13C data. 13E. Binding of [COS(Mu-IL-1R)] cells incubated with [¹²⁵I]-IL-1 α . 13F. Scatchard plot of 13E data.

Figure 14. Construction of Full-length cDNA Clone of Human IL-1R AcP. Schematic representations of the structures of the human IL-1R AcP cDNA inserts in clones #3 and #6 are shown in the upper portion of the figure. Clone #3 contains 5' noncoding sequences, the initiating ATG codon, and a significant portion of the coding region. Clone #6 overlaps with clone #3, containing most of the coding region, the TGA stop codon, and 3' noncoding sequences. The 846 bp *Xba*I/*Bst*XI fragment from clone #3 and the \approx 2700 bp *Bst*XI/*Xba*I fragment from clone #6 were isolated and ligated into the expression vector pEF-BOS as described (Examples 12 and 13). A schematic representation of the resulting cDNA encoding full-length human IL-1R AcP is shown on the bottom line.

Figure 15. Nucleotide Sequence of Human IL-1R AcP. The nucleotide sequence of the open reading frame in the full-length human IL-1R AcP cDNA (Example 13, Figure 14) is shown. The top strand is the coding sequence [SEQ ID NO:1].

Figure 16. Amino Acid Sequence of Human IL-1R AcP. The amino acid sequence of human IL-1R AcP as deduced from translation of the nucleotide sequence in Figure 15 is shown [SEQ ID NO:3]. The signal peptide cleavage site is predicted to occur after Ala-1, resulting in the production of a 550-amino acid mature protein that extends from Ser1 to Val550. The predicted transmembrane domain extends from Leu340 to Leu363.

Figure 17. IL-1 Induction of IL-6 Production in MRC-5 Cells: Inhibition by IL-1 Receptor Antagonist and Anti-Type I IL-1 Receptor Antibody 4C1. Human embryonic lung fibroblast MRC-5 cells (5 X 10⁴ cells; ATCC# CCL-171) were plated into 24-well cluster dishes (No. 3524; Costar) for 24 hrs at 37°C in a humidified incubator. After the 24 hr period, the cells were pretreated with increasing concentrations of either IL-1 receptor antagonist (IL-1RA; 10⁻² to

10³pM), anti-Type I IL-1 receptor antibody 4C1 (10⁻⁴ to 10¹ µg/ml) or nothing for 1 hr at 37°C. At the end of 1 hr, either 5 pM or 100 pM human IL-1β was added and the incubation continued for 24 hrs at 37°C. At the end of the incubation period, 100 µl of cell supernatant
5 was removed from each well and assayed for IL-6 concentration by the Quantikine Human IL-6 Assay Kit (R & D Systems). The data are expressed as the concentration (pg/ml) of IL-6 secreted from the MRC-5 cells in presence of either IL-1β alone or in the presence of IL-1β plus inhibitor. The effect of increasing concentrations of tumor
10 necrosis factor-α (TNFα) on the stimulation of IL-6 secretion from MRC-5 cells was also determined. TNFα was less potent (~500-fold) than IL-1β in stimulating IL-6 secretion from these cells and appeared to be partially dependent on an autocrine secretion of IL-1 by these cells. 17A shows data for IL-1β, TNFα, and inhibition by IL-
15 1ra. 17B shows data for inhibition by mAb 4C1.

Figure 18. Nucleotide Sequence of the Soluble Human IL-1R AcP. The nucleotide sequence of the soluble human IL-1R AcP cDNA is shown. The top strand is the coding sequence [SEQ ID NO:7].

Figure 19. Amino Acid Sequence of the Soluble Human IL-1R AcP. The amino acid sequence of soluble human IL-1R AcP as deduced from translation of the nucleotide sequence in Figure 18 is shown [SEQ ID NO:9].

The present invention is directed to an isolated polynucleotide that encodes a IL-1R AcP (IL-1R AcP) or an active fragment of a IL-1R AcP (i.e. capable of inhibiting the ability of IL-1 to bind to or otherwise activate the IL-1 receptor), in particular a human or
30 murine IL-1R AcP. Examples of such a polynucleotide are the DNA polynucleotide having the sequence [SEQ ID NO: 1], and the DNA polynucleotide encoding the human IL-1R AcP which has the amino acid sequence [SEQ ID NO: 3]. The polynucleotides of this invention may be used as intermediates to produce the protein IL-1R AcP as
35 described below. This protein is useful in treatment of conditions related to IL-1 inflammatory activity. The polynucleotides may themselves be used in treatment by known antisense modalities.

The invention is also directed to IL-1 receptor accessory protein (IL-1R AcP) isolated free of other proteins, or an isolated active fragment of IL-1R AcP. The IL-1R AcP of this invention is a protein or active fragment which inhibits the ability of IL-1 to bind to or
5 otherwise activate the IL-1 receptor.

Part of this invention is a method of obtaining human IL-1R AcP, which method uses as intermediates the following compounds: polynucleotides encoding murine IL-1RAcP, murine IL-1R AcP,
10 antibodies to murine IL-1R AcP, and polynucleotides encoding human IL-1R AcP. From polynucleotides encoding human IL-1R AcP, soluble human IL-1R AcP and antibodies thereof can be obtained. The critical first intermediate for this invention is the isolation of mAbs for the murine IL-1R accessory protein. These mAbs are obtained by
15 immunization with a partially purified preparation of solubilized crosslinked IL-1 α /IL-1R complex from murine 70Z/2 pre-B cells (described in Example 1). The use of the crosslinked ligand-receptor complex was uniquely suitable, since the accessory protein could only be purified as a result of its interaction in such a complex. One of
20 these mAbs (4C5) was then used to isolate a cDNA encoding the murine IL-1R AcP. This murine cDNA was used to obtain a partial genomic clone of the human homologue. A probe derived from the partial genomic clone was then used to isolate the full-length cDNA for human IL-1R AcP.

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As used herein, "polynucleotide" refers to an isolated DNA or RNA polymer, in the form of a separate molecule or as a component of a larger DNA or RNA construct, which has been derived from DNA or RNA isolated at least once in substantially pure form, i.e., free of
30 contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading
35 frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3'

from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

These polynucleotides, e. g. DNA, include those containing one or
5 more of the above-identified DNA sequences and those sequences
which hybridize under stringent hybridization conditions (see, T.
Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring
Harbor Laboratory (1982), pp. 387 to 389) to the DNA sequences. An
example of one such stringent hybridization condition is hybridization
10 at 4 x SSC at 65°C, followed by a washing in 0.1 x SSC at 65°C for an
hour. Alternatively an exemplary stringent hybridization condition is
in 50 % formamide, 4 x SSC at 42°C.

Polynucleotides which hybridize to the sequences for IL-1R AcP
15 under moderate hybridization conditions and which code on
expression for IL-1R AcP peptides having IL-1R AcP biological
properties also encode novel IL-1R AcP polypeptides. Examples of
such non-stringent hybridization conditions are 4 x SSC at 50°C or
hybridization with 30 - 40 % formamide at 42°C. Additional
20 hybridization conditions are mentioned in Example 11. For example, a
DNA sequence which shares regions of significant homology, e. g. sites
of glycosylation or disulfide linkages, with the sequences of IL-1R AcP
and encodes a protein having one or more IL-1R AcP biological
properties clearly encodes a IL-1R AcP polypeptide even if such a
25 DNA sequence would not stringently hybridize to the IL-1R AcP
sequences.

Polynucleotides of this invention were obtained as described in
Examples 7-13 by expressing murine cDNA in eucaryotic cells and
30 screening cell-surface proteins using assays described in Example 7. A
murine cDNA clone was identified which results in the expression of a
protein immunoreactive with mAb 4C5. This cDNA clone was used to
obtain the homologous human genomic clone. Briefly, human genomic
DNA was screened with the intermediate murine IL-1R AcP probe
35 obtained from mouse cells in Example 7. Clones were isolated and
sequenced as described. The partial human genomic clones were then
used as intermediates to screen a human cDNA library and clones

were isolated and sequenced as described to obtain full-length polynucleotides of this invention encoding human IL-1R AcP.

A specific polynucleotide of this invention has the sequence [SEQ ID NO: 1]. Another polynucleotide of this invention encodes the human IL-1R AcP having the amino acid sequence [SEQ ID NO: 3]. Any polynucleotide capable of encoding the amino acid sequence of IL-1R AcP, or specifically [SEQ ID NO: 3] is part of this invention. Another polynucleotide of invention has the sequence [SEQ ID NO: 4].

10

Also part of this invention is a polynucleotide encoding an active fragment of IL-1R AcP. Such polynucleotides are fragments of the polynucleotides provided above (fragmented by known methods such as restriction digestion or shearing) which, when expressed by conventional methods, produce proteins that block IL-1 activity in an IL-1 assay described below. A polynucleotide encoding a soluble IL-1R AcP is a preferred fragment of this invention. An example of such a polynucleotide has the sequence [SEQ ID NO:7].

Polynucleotides encoding the IL-1R AcP and its active fragments are useful as intermediates from which IL-1R AcP and its active fragments are obtained. In addition, these polynucleotides are useful as antisense therapeutics which block the production of IL-1R AcP. Antisense therapeutics are used as described in Akhtar and Ivinson, Nature Genetics 4:215, 1993. RNA or DNA polynucleotides both have these utilities. Antisense polynucleotides which are complementary to [SEQ ID NO:1] or to a fragment of this sequence are part of this invention. Such polynucleotides may be obtained by known methods such as DNA or RNA synthesis to produce a complementary sequence. Thus, any sequence from the polynucleotides of this invention which is capable of hybridizing to DNA or RNA encoding IL-1R AcP under moderately stringent conditions known in the art and which when so hybridized prevents the synthesis of IL-1R AcP is also part of this invention.

30

This invention includes vectors which contain the polynucleotides described herein which encode IL-1R AcP or an active fragment. Any vector known in the art may be used in this capacity,

such as plasmids, phagemids, viral vectors, cosmids and other vectors. The polynucleotides are inserted in the vectors by methods well known in the art of recombinant DNA technology. Expression vectors are a particular example of vectors.

5

As used herein, "expression vector" refers to a vector such as plasmid comprising a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or
10 coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in various eukaryotic expression systems preferably include a signal-
sequence enabling extracellular secretion of translated protein by a
15 host cell. Alternatively, where recombinant protein is expressed without a signal or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

20

Also part of this invention are host cells containing expression vectors containing polynucleotides of this invention, which express IL-1R AcP or active fragments. The polynucleotides are inserted into vectors containing transcriptional regulatory sequences to form
25 expression vectors. These expression vectors are then inserted into host cells by transfection, infection, electroporation, or other well-known methods. Such host cells are capable of producing protein from the expression vectors inserted therein. Other host cells, e.g. yeast, Chinese hamster ovary cells, bacterial cells, can be utilized with the
30 appropriate and suitable expression vectors.

As noted above, this invention is also directed to IL-1 receptor accessory protein (IL-1R AcP) isolated free of other proteins, or an active fragment of IL-1R AcP. The IL-1R AcP of this invention is a
35 protein or active fragment which inhibits the ability of IL-1 to bind to or otherwise activate the IL-1 receptor, especially the Type I IL-1 receptor. Inhibiting activation of the human IL-1 receptor is accomplished by the human IL-1R AcP or active fragments, and has

various effects, in particular reducing inflammation. Thus by means of the IL-1R AcP or active fragment, it is possible to inhibit IL-1 activation of cells and thereby to reduce or alleviate the symptoms associated with inflammation.

5

Active fragments of IL-1R AcP may be obtained by conventional methods for obtaining protein fragments. For example, DNA of this invention may be fragmented by restriction digest or shearing and expressed in host cells by conventional methods to provide fragments
10 of IL-1R AcP. Fragments of the IL-1R AcP may also be obtained by proteolysis of the IL-1R AcP of this invention. Active fragments of this invention are determined by screening for activity using IL-1 assays described below.

15 Soluble IL-1R AcP is an IL-1R AcP fragment of this invention in which deletions of the COOH-terminal sequences result in secretion of the protein into the culture medium. The soluble IL-1R AcP corresponds to all or part of the extracellular region of the IL-1R AcP. Methods for elucidating the COOH terminals and extracellular regions
20 of proteins are well known. The resulting protein preferably retains its ability to interact with IL-1 or the Type I and Type II IL-1R's. Particularly preferred sequences include those in which the transmembrane region and intracellular domain of the IL-1R AcP are deleted or substituted to facilitate secretion of the accessory protein
25 into the culture medium. The soluble IL-1R AcP may also include part of the transmembrane region, provided that the soluble IL-1R AcP is capable of being secreted from the cell. Soluble IL-1R AcP is obtained as described in Examples 14 and 15. A specific soluble IL-1R AcP of this invention has the sequence [SEQ ID NO:9].

30

A preferred example of IL-1R AcP has the amino acid sequence [SEQ ID NO: 3]. The amino acid sequence of the IL-1R AcP as deduced from the cDNA sequence [SEQ ID NO: 1] is shown in Figure 16. Any
35 IL-1R AcP which affects IL-1 binding as described above, is included in this invention, such as an analogue having the sequence of [SEQ ID NO: 3], in which one or more side groups have been modified in a known manner, by attachment of compounds such as polyethylene glycol, or by incorporation in a fusion protein (with other protein

sequences such as immunoglobulin sequences), for example, or proteins whose activity has otherwise been maintained or enhanced by any such modification. Also included are proteins which inhibit IL-1 binding to the IL-1 receptor and have essentially the sequence
5 [SEQ ID NO:3] with one or more amino acids added, deleted, or substituted by known techniques such as site-directed mutagenesis. The change in amino acids is limited and conservative so as to maintain the identity of the protein as an IL-1R AcP with all or part of its activity as described, or enhanced activity. Means for
10 determining IL-1 inhibiting activity are described in Examples 5, 6, 16 and include inhibition of IL-1 binding to IL-1 receptor, inhibition of lymphocyte proliferation or kappa light chain expression, and decrease of IL-1 induced IL-6 expression.

15 IL-1R AcP isolated free of other proteins may be obtained from the polynucleotides of this invention which encode IL-1R AcP. For example, IL-1R AcP may be obtained by conventional methods of expressing a polynucleotide provided herein encoding IL-1R AcP, preferably the DNA of [SEQ ID NO: 1] or [SEQ ID NO: 7] in a host cell,
20 and isolating the resulting protein. Once the IL-1R AcP is obtained, the protein can be isolated free of other proteins by conventional methods. These methods include but are not limited to purification or antibody affinity columns with the antibodies of this invention, chromatography on ion exchange or gel filtration columns, purification
25 by high performance liquid chromatography, and purification with an IL-1 affinity column.

IL-1R AcP may be stabilized by attaching a polyalkylene glycol polymer by known methods. Polyalkylene glycol includes poly-
30 ethylene glycol, and other polyalkylene polymers which may be branched or unbranched. The polymers may be directly linked to the protein, or may be linked by means of linking groups connecting for example the COOH of the polymer to the NH₂ of a lysine on the protein.

35

IL-1R AcP of this invention may be used directly in therapy to bind or scavenge IL-1, thereby providing a means for regulating and preventing the inflammatory or immunological activities of IL-1. In

its use to prevent or reverse pathologic responses, soluble IL-1R AcP or antibodies to the IL-1R AcP can be combined with other cytokine antagonists such as antibodies to the IL-2 receptor, soluble TNF receptor, the IL-1 receptor antagonist, soluble IL-1 receptor and the like. In addition, isolated IL-1R AcP of this invention is useful in raising antibodies to IL-1R AcP which are themselves useful in therapy. Raising such antibodies is made feasible because this invention makes available IL-1R AcP in sufficient amounts for antibody production.

10

Thus, this invention is also directed to antibodies to human IL-1R AcP. Murine or rat monoclonal antibodies to human IL-1R AcP are obtained as in Example 15. These antibodies are obtained by immunization with purified or partially purified amounts of human IL-1R AcP, which is obtained after expression of the recombinant full-length or soluble human IL-1R AcP using the DNA's of this invention. The human IL-1R AcP cDNA's were isolated using the murine IL-1R AcP DNA of this invention which was isolated with the unique mAb 4C5 described in Examples 2 and 3. For the murine or rat mAbs to human IL-1R AcP, hybridoma techniques well known in the art may then be used to obtain hybridomas to generate mAbs. Chimeric antibodies and humanized antibodies may be obtained from these rodent antibodies using known methods. (Brown et al., Proc. Natl. Acad. Sci. USA 88: 2663, 1991; WO 90/7861, EP 620276) or by producing heterodimeric bispecific antibodies (Kostelny et al., J. Immunol. 148: 1547, 1992).

Antibodies to human IL-1R AcP of this invention bind specifically to human IL-1R AcP and prevent activation of the IL-1 receptor complex by IL-1. This activity may be determined by assays as described herein. Specifically, biological assays include screens based on the ability of the antibody to inhibit the proliferation of IL-1-responsive cells or the IL-1-induced secretion of prostaglandin E₂ and IL-6. Such assays can be carried out by conventional methods in cell biology. Suitable cells for these assays include splenic B cells, cell lines such as the human B cell line RPMI 1788 (Vandenabeele et al., J. Immunol. Meth. 135: 25, 1990), and human fibroblasts such as the human lung fibroblast line MRC-5 (Chin et al., J. Exp. Med. 165: 70,

1987). Methods for such assays using mouse cells are found in Examples 1, 2, 5, and 6. For example an *in vivo* assay may be used, which measures inhibition of IL-1 induced IL-6 production in mice. These assays may be performed using human cells to effectively
5 screen for the desired activity using the same techniques provided in the Examples. A preferred antibody has a binding affinity to the IL-1 receptor accessory complex of about K_D 0.1 nM to about K_D 10 nM, as determined by conventional methods (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949).

10

The antibodies of this invention may be administered by known methods to relieve conditions caused by the presence of IL-1. In particular, the antibodies of this invention are useful in reducing inflammation. These antibodies to the IL-1R AcP can be administered,
15 for example, for the purpose of suppressing inflammatory or immune responses in a human. A variety of diseases or conditions caused by inflammatory processes (e.g. rheumatoid arthritis, inflammatory bowel disease, and septic shock) or by immune reactions (e.g. Type I diabetes, transplant rejection, psoriasis, and asthma) are associated
20 with elevated levels of IL-1 (Dinarello and Wolff, New Engl. J. Med. 328: 106, 1993). Treatment with antibodies that inhibit IL-1 interaction with the IL-1R AcP may therefore be used to effectively suppress inflammatory or immune responses in the clinical treatment of acute or chronic diseases such as rheumatoid arthritis,
25 inflammatory bowel disease, and Type I diabetes. In addition, antibodies are useful in the treatment of certain cancers, such as acute and chronic myelogenous leukemia (Rambaldi et al., Blood 78: 3248, 1991; Estrov et al., Blood 78: 1476, 1991).

30 Included in this invention are antibodies to murine IL-1R AcP, specifically 4C5, 2B5, 3F1, 4C4, 24C5, 4D4 (see Table 1) and 1D2, 2D6, 2E6, 1F6, 2D4, 2F6, 3F5, and 4A1 (see Table 2). These antibodies are useful to obtain human IL-1R AcP, as described.

35 As noted above, antibodies may be produced naturally by appropriate cells, or may be produced by recombinant expression vectors that modify the antibody proteins, e.g. by humanizing the antibody (Brown et al., Proc. Natl. Acad. Sci. USA 88: 2663, 1991) or

by producing heterodimeric bispecific antibodies (Kostelny et al., J. Immunol. 148: 1547, 1992; WO 90/7861, EP 620276) that can recognize both the accessory protein and the Type I or Type II IL-1R.

5 The dose ranges for the administration of the IL-1R AcP and fragments thereof or of antibodies to the IL-1R AcP or antisense polynucleotides may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for
10 example, blocking the activity of endogenous IL-1 to cells responsive to IL-1. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter-indications, if any,
15 immune tolerance and other such variables, to be adjusted by the individual physician. The IL-1R AcP and fragments thereof or antibodies to this protein or antisense polynucleotides can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally,
20 intramuscularly, or subcutaneously.

This invention includes pharmaceutical compositions comprising the proteins and/or antibodies of this invention in amounts effective to reduce inflammation, and a pharmaceutically acceptable carrier
25 such as the preparations and vehicles described below. Such compositions may include other active compounds if desired. For the proteins, an example of an effective amount is in the range of about 4 to about 32 mg/meter². For antibodies, an example of an effective amount is in the range of about 0.1 to about 15 mg/kg body weight.

30

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters
35 such as ethyl oleate. Aqueous carriers include water, alcoholic/ aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or

fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds., 1980.

The following Examples are provided to further describe the invention and are not intended to limit it in any way.

10

Example 1

Methods

15 Preparation, Screening and Purification of Hybridoma Antibodies

Lewis Rats (Charles River Laboratories) were immunized by the intraperitoneal (i.p) route with detergent solubilized preparations of human IL-1 α (Gubler et al., J. Immunol. 136: 2492, 1986), affinity cross-linked to IL-1R from murine 70Z/3 pre-B cells (ATCC #TIB 158). For the primary immunization, the rats received solubilized IL-1 α /IL-1R complex (0.4 ml) that was prepared and purified from 1×10^{11} 70Z/3 cells (Chizzonite et al., Proc. Natl. Acad. Sci. USA 86: 8029, 1989) and emulsified in Freund's Complete Adjuvant at a 1:2 ratio and injected i.p. (described below). Six weeks later, the rats received solubilized IL-1 α /IL-1R complex (0.3 ml) that was prepared and purified from 2.25×10^{11} cells and emulsified in Freund's Complete Adjuvant at a ratio of 1:2 and injected in each hind foot pad and i.p. Sera were collected from the rats at 2 and 6 weeks after the last immunization and tested for activity that blocked [125 I]-IL-1 β binding to IL-1R on 70Z/3 cells. Four months after the last immunization, one rat was immunized with the following amounts of solubilized IL-1 β /IL-1R complex in preparation for splenocyte isolation: 0.1 ml (prepared and purified from 8×10^{10} cells) emulsified at a 1:4 ratio with Freund's Complete Adjuvant and injected in each hind foot pad and subcutaneous (s.c.) in each hind limb, and 0.9 ml (prepared and purified from 7.4×10^{11} 70Z/3 cells) injected intravenous (i.v.) and i.p. Two days later, the rat was immunized with solubilized IL-1 α /IL-1R complex (0.5 ml; prepared

and purified from 2×10^{11} 70Z/3 cells) mixed with phosphate buffered saline (PBS), pH 7.4 (0.5 ml) and injected s.c. in each hind limb. Two days after this last immunization, spleen cells were isolated from the rat and fused with SP2/0 cells (ATCC CRL 1581) at a ratio of
5 1:1 (spleen cells:SP2/0 cells) with 35% polyethylene glycol (PEG 4000, E. Merck) according to a published procedure (Fazekas et al., J. Immunol. Meth. 35: 1, 1980). The fused cells were plated at a density of 3×10^5 cells/well/ml in 48 well plates in IMDM supplemented with 15% FBS, glutamine (2 mM), beta-mercaptoethanol (0.1 mM),
10 gentamicin (50 μ g/ml), HEPES (10 mM), 5% ORIGIN hybridoma cloning factor (IGEN, Inc.), 5% P388D1 supernatant (Nordon et al. J. Immunol. 139: 813, 1987) and 100 Units/ml recombinant human IL-6 (Genzyme).

15 Hybridoma supernatants were screened for inhibitory and non-inhibitory antibodies specific for an IL-1R AcP and the Type II IL-1R in four assays: 1) for inhibitory antibodies: inhibition of [125 I]-IL-1 β binding to 70Z/3 and EL-4 thymoma cells (described below), 2) for non-inhibitory antibodies: immunoprecipitation of solubilized complex
20 of [125 I]-IL-1 β crosslinked to Type II IL-1R, 3) for inhibitory antibodies specific for IL-1R AcP or Type II IL-1R: inhibition of [125 I]-IL-1 β and [125 I]-IL-1 α binding to cells expressing recombinant Type I and Type II IL-1Rs, and 4) to eliminate any antibodies specific for IL-1: immunoprecipitation of [125 I]-IL-1 α and
25 [125 I]-IL-1 β . Hybridoma cell lines secreting antibodies specific for Type II IL-1R and the IL-1R AcP were cloned by limiting dilution. Antibodies were purified from large scale hybridoma cultures or ascites fluids by affinity chromatography on protein G bound to Sepharose 4B fast flow according to the manufacturer's protocol
30 (Pharmacia).

Cultured Cells and Biological Assays

Mouse EL-4.IL-2 thymoma cells (TIB 181) and D10.G4.1 (TIB
35 224) cells were maintained as previously described (Kilian et al., J. Immunol. 136: 1, 1986). Mouse 3T3L1 (CL 173) and 70Z/3 pre-B (TIB 158) cells were maintained in IMDM containing 5% fetal bovine serum

in 600 cm² dishes. The above cells were obtained from the American Type Culture Collection and the ATTC numbers are in parenthesis.

The biological activity of unlabeled IL-1 and [¹²⁵I]-IL-1
5 proteins were evaluated in the murine D10 proliferation assay (Kaye et al., J. Exp. Med. 158: 836, 1983).

Labeling of IL-1 and Purified Monoclonal Antibodies with ¹²⁵I

10 Recombinant murine IL-1 α , human IL-1 α and human IL-1 β were purified as previously described (Kilian et al., J. Immunol. 136: 1, 1986; Gubler et al., J. Immunol 136: 2492, 1986) except that murine IL-1 α was prepared in 25 mM Tris-HCl, 0.4 M NaCl. Protein
15 determinations were performed by BCA protein assay (Pierce Chemical Co., Rockford, IL). Human IL-1 α human IL-1 β , murine IL-1 α , murine IL-1 β and purified IgG were labeled with ¹²⁵I by a modification of the Iodogen method (Pierce Chemical Co.). Iodogen was dissolved in chloroform and 0.05 mg dried in a 12 x 15 mm borosilicate glass tube. For radiolabeling, 1.0 mCi Na[¹²⁵I]
20 (Amersham, Chicago, IL) was added to an Iodogen-coated tube containing 0.05 ml of Tris-iodination buffer (25 mM Tris-HCl pH 7.5, 0.4 M NaCl, 1 mM EDTA) and incubated for 4 min at room temperature. The activated ¹²⁵I solution was transferred to a tube containing 0.05 to 0.1 ml IL-1 (5-13 μ g) or IgG (100 μ g) in Tris-
25 iodination buffer and the reaction was incubated for 5-8 min at room temperature. At the end of the incubation, 0.05 ml of Iodogen stop buffer (10 mg/ml tyrosine, 10% glycerol in Dulbecco's PBS, pH 7.4) was added and reacted for 3 min. The mixture was then diluted with 1.0 ml Tris-iodination buffer, and applied to a Bio-Gel P10DG desalting
30 column (BioRad Laboratories) for chromatography. The column was eluted with Tris-iodination buffer, and fractions (1 ml) containing the peak amounts of labeled protein were combined and diluted to 1 x 10⁸ cpm/ml with 1% BSA in Tris-iodination buffer. The TCA precipitable radioactivity (10% TCA final concentration) was typically
35 in excess of 95% of the total radioactivity. The radiospecific activity was typically 2000 to 3500 cpm/fmol for purified antibodies and 3500 to 4500 cpm/fmole for IL-1.

Mouse IL-1 Receptor Binding Assays

Binding of radiolabeled IL-1 to mouse cells grown in suspension culture was measured by a previously described method (Kilian et al., J. Immunol. 136: 1, 1986). Briefly, cells were washed once in binding buffer (RPMI-1640, 5% FBS, 25 mM HEPES, pH 7.4), resuspended in binding buffer to a cell density of 1.5×10^7 cells/ml and incubated (1.5×10^6 cells) with various concentrations of [125 I]-IL-1 (5-1000 pM) at 4°C for 3-4 hrs. Cell bound radioactivity was separated from free [125 I]-IL-1 by centrifugation of the assay mixture through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 : A.H. Thomas, and Silicone Oil AR 200 : Gallard-Schlessinger) at 4°C for 90 sec at 10,000 x.g. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non-specific binding was determined by inclusion of 50 nM unlabeled IL-1 in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed by using the non-linear regression programs EBDA, LIGAND and Kinetic (Munson and Rodbard, Anal. Biochem 107: 220, 1980) as adapted for the IBM personal computer by McPherson (McPherson, J. Pharmacol. Methods 14: 213, 1985) from Elsevier-BIOSOFT.

The binding of radioiodinated IL-1 proteins to adherent cells was performed by incubating cells and ligands in a 24 or 12 well plate at 4°C on a rocker platform for 4 hrs in binding buffer (24). Monolayers were then rinsed 3 times with binding buffer at 4°C, solubilized with 0.5 ml 1% SDS and the released radioactivity counted in a gamma counter. Non-specific binding was determined in the presence of 50 nM unlabeled IL-1. Analysis of the binding data was performed as described above.

Equilibrium Binding of [125 I]-labeled Monoclonal Antibodies to Murine Cells

Murine cells were washed once in binding buffer (RPMI 1640, 5% FBS, 25 mM Hepes, pH 7.4) and resuspended in binding buffer to a cell density of 1.5×10^7 cells/ml. Cells (1.5×10^6) were incubated with various concentrations of [125 I]-specific IgG (.005 to 2 nM) at

room temperature for 1.5-2 hrs. Cell bound radioactivity was separated from free [125 I]-labeled antibody by centrifugation of the assay mixture through 0.1 ml silicone oil at 4°C for 90 seconds at 10,000 x g. The tip containing the cell pellet was exercised, and cell bound radioactivity was determined in a gamma counter. Non-specific binding was determined by inclusion of 100 nM unlabeled antibody in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed as described above for IL-1 binding to cells.

10

Antibody Mediated Inhibition of [125 I]-IL-1 Binding to Murine Cells Bearing Type I or Type II IL-1 Receptors

The ability of hybridoma supernatant solutions, purified IgG, or antisera to inhibit the binding of [125 I]-IL-1 proteins to murine cells bearing IL-1 receptor was measured as follows: serial dilutions of culture supernatants, purified IgG or antisera were mixed with cells ($1-1.5 \times 10^6$ cells) in binding buffer (RPMI-1640, 5% FBS, 25 mM Hepes, pH 7.4) and incubated on an orbital shaker for 1 hour at room temperature. [125 I]-IL-1 (1×10^5 cpm; 25 pM) was added to each tube and incubated for 3-4 hours at 4°C. Non-specific binding was determined by inclusion of 50 nM unlabeled IL-1 in the assay. Incubations were carried out in duplicate or triplicate. Cell bound radioactivity was separated from free [125 I]-IL-1 by centrifugation of the assay through 0.1 ml of an oil mixture as described above. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter.

Affinity Cross-linking and Purification of Solubilized [125 I]-IL-1 α /IL-1R Complexes

Affinity cross-linking of radioiodinated IL-1 proteins to cells was performed as described (Riske et al., J. Biol. Chem. 266: 11245, 1991) with minor modifications. Briefly, cells (1.5×10^7 cells/ml) were incubated with radiolabeled IL-1 (60-300 fmoles/ml) in the presence or absence of 50 nM unlabeled IL-1 for 4 hrs at 4°C in binding buffer. The cells were then washed with ice cold PBS, pH 8.3 (25 mM sodium phosphate, pH 8.3, 0.15 M NaCl, 1 mM MgCl₂),

resuspended at a concentration of 5×10^6 cells/ml in PBS, pH 8.3. Disuccinimidyl suberate (DSS) or bis(sulfosuccinimidyl)suberate (BS3) (Pierce Chemical Co.) in dimethyl sulfoxide was added to a final concentration of 0.4 mM. Incubation was continued for 30-60 min at 4°C with constant agitation. The cells were washed with ice cold 25 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 5 mM EDTA and solubilized at $0.5-1 \times 10^8$ cells/ml in solubilization buffer (50 mM sodium phosphate, pH 7.5, containing either 8 mM CHAPS or 1% Triton X-100, 0.25 M NaCl, 5 mM EDTA, 40 µg/ml phenylmethylsulfonyl fluoride, and 0.05% NaN_3) for 1 hr at 4°C. The detergent extract was centrifuged at $120,000 \times g$ for 1 hr at 4°C to remove nuclei and other debris. The extracts were directly analyzed by SDS-PAGE on 8% pre-cast gels (NOVEX) followed by autoradiography. Alternatively, the extracts were immunoprecipitated with antibody bound to Gamma-Bind G Plus (Pharmacia). The precipitated proteins were released by treatment with Laemmli sample buffer (Laemmli, Nature 227: 680, 1970), separated by SDS-PAGE and analyzed by autoradiography.

Preparation of the solubilized crosslinked complex of IL-1 α /IL-1R that was used as the immunogen was performed as described above with minor modifications. Briefly, 70Z/3 cells ($0.5-1.0 \times 10^8$ cells/ml) were incubated with IL-1 α (0.5 to 1.0 nM) for 4 hrs at 4°C in binding assay buffer. The cells were then washed with ice cold PBS, pH 8.3, resuspended at a concentration of 5×10^7 cells/ml in PBS, pH 8.3 and bis(sulfosuccinimidyl)suberate (BS3) (Pierce Chemical Co.) in dimethyl sulfoxide was added to a final concentration of 0.4 mM. Incubation was continued for 30-60 min at 4°C with constant agitation. The quenching of the affinity crosslinking procedure and the detergent solubilization of the cells was as described above.

For purification of the solubilized IL-1 α /IL-1R complex that was used as the immunogen, the detergent extract of 70Z/3 cells was applied to an affinity column (10 ml) of goat anti-human IL-1 α immobilized on crosslinked beaded agarose (Affi-Gel 10, BioRad Laboratories). The goat anti-human IL-1 α affinity column was prepared according to the manufacturer's instructions at a density of 1 mg of IgG/ml of packed gel. After application of the detergent extract, the column was washed with 10 column volumes of

solubilization buffer without Chaps or Triton X-100 or until the absorbance at 280nm was at baseline. The column was then eluted with 3 M potassium thiocyanate, 25 mM sodium phosphate, pH 7.5, 5 mM EDTA, 40 µg/ml phenylmethylsulfonyl fluoride, and 0.05% NaN₃.

- 5 The proteins eluted from the affinity column were concentrated 10 to 100 fold and used for immunization.

Immunoblot Analysis of Proteins Solubilized from Murine Cells

- 10 Murine 70Z/3 and EL-4 cells were washed 3 times with ice-cold PBS and solubilized at $0.5 - 1 \times 10^8$ cells/ml in solubilization buffer that contained either 8 mM CHAPS or 1% Triton X-100 and 1 mg/ml BSA for 1 hr at 4°C. The extracts were centrifuged at 120,000 x g for 45 min at 4°C to remove nuclei and other debris. The extracts were
- 15 incubated with either 4C5 (anti-IL-1R AcP obtained as described in Example 2), 12A6 (anti-Type I IL-1R obtained as described in Chizzonite et al., Proc. Natl. Acad. Sci. USA 86:8029, 1989) or control antibody bound to protein-G immobilized on crosslinked agarose (Gamma Bind G Plus, Pharmacia). The precipitated proteins were
- 20 released by treatment with 0.1 M glycine pH 2.3, neutralized with 3M Tris, mixed with 1/5 volume of 5X Laemmli sample buffer, and separated by SDS/PAGE on 8% pre-cast acrylamide gels (NOVEX). The separated proteins were transferred to nitrocellulose membrane (0.2 µM) for 16 hours at 100 volts in 10 mM Tris-HCl pH 8.3, 76.8 mM
- 25 glycine, 20% methanol and 0.01% SDS. The nitrocellulose membrane was blocked with BLOTTO (50% w/v nonfat dry milk in PBS + .05% Tween 20) and duplicate blots were probed with [¹²⁵I]-4C5 IgG (1×10^6 cpm/ml in 8mM CHAPS, PBS, 0.25 M NaCl, 10% BSA and 5 mM EDTA) with and without unlabeled 4C5 IgG (67nM).

30

Expression of Murine Recombinant Type I and Type II IL-1 Receptors and IL-1R AcP in COS Cells and Determination of [¹²⁵I]-labeled 4C5, 35F5 and IL-1 Binding

- 35 COS cells ($4-5 \times 10^7$) were transfected by electroporation with 25 µg of plasmid DNA expressing recombinant murine IL-1R proteins or IL-1R AcP in a BioRad Gene Pulser (250 µF, 250 volts) according to the manufacturer's protocol. The cells were plated in a 600 cm²

culture plate, harvested after 72 hours by treatment with No-Zyme (JRH Biologics) and scraping, washed and resuspended in binding buffer. Transfected cells ($4-8 \times 10^4$) were incubated with increasing concentrations of [125 I]-labeled 4C5, 35F5 or IL-1 proteins at 4°C for 3 hrs. Cell bound radioactivity was separated from free [125 I]-labeled antibody or IL-1 as described above.

Kappa Light Chain Expression by 70Z/3 Cells in Response to IL-1:
Inhibition by Monoclonal Antibodies 35F5, 4E2 and 4C5

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70Z/3 cells (1×10^5 /ml in RPMI 1640, supplemented with 10% FBS, β -mercaptoethanol and gentamicin) were incubated with and without 100 U/ml (0.19 nM) of human recombinant IL-1 α or IL-1 β for 24 hrs or 48 hrs. The cells were preincubated for one hour before the addition of IL-1 with 30 μ g/ml of the indicated antibodies in a total volume of 0.5 ml. An additional 0.5 ml of medium containing the IL-1 or medium alone was added to the wells for a final concentration of 15 μ g/ml (100 nM) antibodies. The cells were washed once after culture and stained with either a control rat antibody conjugated with FITC or rat anti-mouse kappa light chain antibody conjugated with FITC (Tago, Burlingame, Ca). The cells were then analyzed for kappa light chain expression on a FACScan flow cytometer (Becton-Dickinson).

25 Proliferation of Murine Splenic B cells in Response to IL-1: Inhibition by Monoclonal Antibodies 35F5, 4C5 and 4E2.

Splenic B cells were purified by treating splenocytes isolated from C57BL/6 mice with anti-Thy1.2 antibody and rabbit complement, followed by two sequential passages through a Sephadex G10 (Pharmacia) columns. B cells (5×10^5 cells) were treated with goat anti-mouse IgM (1 μ g/ml) (ZYMED) and dibutyryl cAMP (10^{-3} M) in a final volume of 200 μ l of RPMI 1640 media supplemented with 10% FBS, β -mercaptoethanol and gentamicin. Splenic B cells were treated with and without IL-1 (100 U/ml) and with and without antibodies 35F5, 4C5 and 4E2. The cells were incubated for two days in the presence of the various reagents and then pulsed with 0.5 μ Ci tritiated thymidine, incubated for an additional 6 hrs, and harvested.

Proliferation of Murine D10.G4.1 Cells in Response to IL-1: Inhibition by Monoclonal Antibodies 4C5 and 35F5 and Human IL-1ra

5 D10.G4.1 helper T cells were maintained as described (Kaye et al., J. Exp. Med. 158: 836, 1983; McIntyre et al., J. Exp. Med. 173: 931, 1991) and stimulated with IL-1 as previously described (McIntyre et al., J. Exp. Med. 173: 931, 1991). Cells (1×10^5 in 200 μ l) were incubated with 0.2 pM IL-1 in RPMI 1640 containing 5% FBS,
10 β -mercaptoethanol (5×10^{-5} M), gentamicin (8 μ g/ml), 2 mM L-glutamine, 2.5 μ g/ml concanavalin A and the indicated concentrations of antibodies or human IL-1 receptor antagonist (IL-1ra). The cultures were incubated for two days, pulsed with 0.5 μ Ci tritiated thymidine and harvested 16 hrs later.

15

In Vivo Induction of Serum IL-6 by IL-1: Inhibition by Monoclonal Antibodies 35F5 and 4C5

The induction of serum IL-6 by IL-1 was performed as
20 previously described (McIntyre et al., J. Exp. Med. 173: 931, 1991). Briefly, C57BL/6 mice were pretreated (i.p) with 250 μ g of antibody at 4 hrs and 10 min before administration of IL-1 α or IL-1 β (0.3 μ g/mouse, s.c.). Sera were collected from the mice 2 hrs after administration of IL-1 and analyzed for IL-6 concentration by a
25 modification of the B9 hybridoma cell bioassay as described (Aarden et al., Eur. J. Immunol. 17: 1411, 1987).

The rat anti-mouse IL-1 accessory protein monoclonal antibody 4C5 was prepared, characterized and generated as follows:

30

Example 2

Preparation, Characterization and Identification of Monoclonal Antibodies Specific for IL-1R AcP and Type II IL-1R

35

In the course of preparing antibodies to the Type II IL-1 receptor, antibodies to an unexpected, novel component of the IL-1 receptor complex were detected. Since murine 70Z/3 cells express

almost exclusively the Type II IL-1R, immunization of rats with the purified crosslinked IL-1 α /IL-1R complex solubilized from these cells was the initial strategy pursued to develop monoclonal anti-Type II IL-1R antibodies. Rats immunized with this solubilized IL-1 α /IL-1R complex developed serum antibodies that blocked [¹²⁵I]-IL-1 β binding to 70Z/3, indicating the presence of blocking antibodies specific for the Type II IL-1R. The serum samples also contained antibodies that immuno-precipitated the [¹²⁵I]-IL-1 β /IL-1R complex solubilized from 70Z/3 cells, indicating the presence of non-blocking anti-Type II IL-1R antibodies. [¹²⁵I]-IL-1 β was used for the IL-1R binding and immunoprecipitation assays to eliminate identification of antibodies specific for IL-1 α instead of the Type II receptor.

Hybridomas resulting from the fusion of splenocytes isolated from the immunized rat were screened for antibodies that blocked IL-1 β binding to both 70Z/3 (Type II receptor bearing) and EL-4 (Type I receptor bearing) cells. Antibodies that block binding only to 70Z/3 cells were identified and eliminated from further analysis because they are antibodies to Type II IL-1R, and antibodies that blocked binding only to EL-4 cells were identified and eliminated from further analysis because they are antibodies to Type I IL-1R. Antibodies that blocked IL-1 binding to both cell types are specific for the IL-1R AcP.

From the initial fusion, seven antibodies were identified that blocked IL-1 β binding to 70Z/3 cells (Table 1). Six of these antibodies (2B5, 4C5, 3F1, 4C4, 24C5, and 4D4) blocked IL-1 β binding to both 70Z/3 and EL-4 cells. These antibodies did not block IL-1 β binding to CHO cells expressing murine recombinant Type I IL-1R, and were therefore specific for an IL-1R AcP. One antibody, 4E2, only blocked IL-1 β binding to 70Z/3 cells, indicating that it was specific for the Type II IL-1R.

The initial fusion was also screened for non-blocking antibodies that were specific for either the IL-1R AcP or the Type II IL-1R. Eight antibodies (1D2, 2D6, 2E6, 1F6, 2D4, 2F6, 3F5 and 4A1) immuno-precipitated the IL-1 β /IL-1R complex solubilized from 70Z/3 cells (Table 2). These antibodies also immunoprecipitated the IL-1 β /IL-1R

complexes solubilized from two other Type II IL-1R bearing murine cell lines, AMJ2C11 and P388D1. Seven of these antibodies also immunoprecipitated the IL-1 β /IL-1R complex solubilized from EL-4 cells, demonstrating that they recognized an IL-1R AcP. One antibody, 5 1F6, did not bind to the IL-1 β /IL-1R complex solubilized from EL-4

1. Inhibition of [125 I]-IL-1 β binding to cell lines, 7OZ/3, AMJ2C11, P388D1, EL-4 and CHO (Mu Type I IL-1R) by antibodies was described in Example 1.
2. Immunoprecipitation of [125 I]-labelled recombinant IL-1 proteins was as described in Example 1.
3. rHuIL-1 α = human recombinant IL-1 α .
rHuIL-1 β = human recombinant IL-1 β .
rMuIL-1 α = murine recombinant IL-1 α .
4. ++ and +; antibody blocks [125 I]-IL-1 β Binding.
5. -; antibody was negative in the assay.

1. As described in Table 1.
2. Immunoprecipitation of the complex of [125 I]-I-IL-1 β /IL-1R solubilized from the indicated cell lines.
3. Immunoprecipitation of the complex of [125 I]-IL-1 β affinity crosslinked to the soluble murine Type I IL-1R expressed in a baculovirus system.
4. Immunoprecipitation of [125 I]-labelled soluble Type I IL-1R expressed in either baculovirus [125 I-sMUL-1R (bv)] or Cos cell [125 I-sMsR(Cos)] expression systems.

cells, indicating it was a non-blocking Type II IL-1R antibody. To confirm that these antibodies did not bind to the Type I IL-1R, they were tested in immunoprecipitation assays with murine soluble Type I IL-1R (Table 2). None of these antibodies immunoprecipitated the
5 complex of [125 I]-IL-1 β crosslinked to recombinant soluble Type I IL-1R ([125 I]-sMsR[bv]). They also did not immunoprecipitate [125 I]-labeled soluble Type I receptor produced either in a baculovirus/insect cell expression system or in a COS cell expression system (Table 2).

10

Since the rats were immunized with the solubilized IL-1 α /IL-1R complex, antibodies in the rat serum were also detected that recognized IL-1 α . Each monoclonal antibody was tested in immunoprecipitation assays with [125 I]-labeled murine and human
15 IL-1 proteins to confirm that they did not bind to IL-1. All 15 antibodies (Tables 1 and 2) were negative in these assays.

Example 3

20 Characterization of Murine IL-1Rs and IL-1R AcP by Reactivity with Anti-Type I (35F5), Type II (4E2) and Accessory Protein (4C5) Monoclonal Antibodies

Following the initial identification and characterization of the
25 antibodies described above, 4C5, a putative blocking IL-1R AcP (IL-1R AcP) antibody, and 4E2, a blocking Type II IL-1R antibody, were chosen as probes for the further study of the IL-1R AcP. A previously identified and characterized anti-Type I IL-1R antibody, 35F5, was also included in this study (Chizzonite et al., Proc. Natl. Acad. Sci. USA
30 86: 8029, 1989), McIntyre et al., J. Exp. Med. 173: 931, 1991).

These three antibodies were used to identify the presence of Type I and Type II IL-1R's and IL-1R AcP on various murine cells. Equilibrium binding assays with [125 I]-labeled mAb 4C5
35 demonstrated the presence of IL-1R AcP on murine cells bearing predominately Type I (EL-4 cells) or Type II (70Z/3 cells) receptors (Figures 1 and 2). Other cells bearing predominately Type I (3T3L1 cells) or Type II (P388D1 cells) receptors also expressed IL-1R AcP

(Table 3). Cells (S49.1) that do not express either Type I or Type II IL-1R AcP did not express IL-1R AcP, indicating a link between expression of IL-1R and IL-1R AcP. During its initial characterization, mAb 4C5 blocked [¹²⁵I]-human IL-1 β binding to
5 both EL-4 and 70Z/3 cells. Further studies established

Table 3

Equilibrium Binding of Radiolabelled IL-1, 4C5 and 4E2 to Murine Cells Expressing Predominantly Type I or Type II IL-1Rs

Ligand	CELL LINE									
	EL-4 ⁷	S49.1 ⁸	3T3L1 ⁷	7OZ/3 ⁴		P388D1 ⁴		COS II ⁴		
	K _D ²	S/C ³	K _D	S/C	K _D	S/C	K _D	S/C	K _D	S/C
[¹²⁵ I]-IL-1										
rMuIL-1α	.05	1200	.008	1640	.2	1500	.19	380	.21	1950
rHuIL-1α	.05	1200							.33	1200
rHuIL-1β	.1	1200								
[¹²⁵ I]-4C5	1.2	2800							NSB	
			.93	19200	1.4	3000	.77	1870		
			.61	14200						
[¹²⁵ I]-4E2	NSB		NSB		1.2	1900	ND		ND	
			NSB							

1.	Abbreviations of IL-1 proteins as in Table 1.
2.	K_D = equilibrium dissociation constant (nM).
3.	S/C = binding sites per cell.
4.	Cells expressing murine natural or recombinant Type II IL-1R.
5.	NSB = no specific binding of the radiolabelled ligand.
6.	ND = not determined.
7.	Cells expressing murine natural Type I IL-1R.
8.	Cells not expressing either murine Type I or Type II IL-1Rs.

that mAb 4C5 also inhibited the binding of radiolabeled human IL-1 α (Fig. 3), murine IL-1 α and IL-1 β to 70Z/3 cells (Table 4). Similar to its inhibition of [125 I]-human IL-1 β binding to EL-4 cells, 4C5 also blocked [125 I]-murine IL-1 β binding to these cells (Table 4).

5 However, 4C5 did not block either radiolabeled human IL-1 α (Fig. 4) or murine IL-1 α (Table 4) binding to EL-4 cells. Moreover, 4C5 did not block the binding of [125 I]-labeled IL-1 proteins to CHO or COS cells expressing murine recombinant Type I or Type II receptors. The anti-Type I receptor antibody, 35F5, and the anti-Type II receptor

10 antibody, 4E2, inhibited both IL-1 α and IL-1 β binding to their respective IL-1 receptors, regardless of whether the receptors were the natural or recombinant forms (Table 4). The IC₅₀s for 4C5-mediated inhibition of IL-1 binding to EL-4 and 70Z/3 cells were at least 1000-fold lower than IC₅₀s for inhibition of binding to cells

15 expressing recombinant Type I or Type II receptors (Table 5). These IC₅₀ data suggested two conclusions: 1) mAb 4C5 did not crossreact to any significant extent with Type I or Type II IL-1R's, and 2) the difference in the ability of 4C5 to block IL-1 β , but not IL-1 α , binding to natural IL-1R's was unrelated to the affinity of the antibody.

20

Example 4

Determination of the Size of the IL-1R AcP Recognized by Monoclonal Antibody 4C5

25

The approximate molecular size of the cell surface protein recognized by mAb 4C5 on EL-4 cells was determined by affinity chromatography and immunoblotting to be approximately 90 kDa (Fig. 5). Detergent extracts prepared from EL-4 cells were purified on a lentil lectin

30 affinity matrix followed by affinity chromatography on either an anti-Type I receptor antibody (7E6), murine IL-1 α (Ma) or 4C5 affinity gel. The proteins eluted from each affinity column were treated with Laemmli sample buffer, separated by SDS-PAGE on 8% gels and transferred to nitrocellulose membrane. The proteins immobilized on

35 the nitrocellulose were probed with [125 I]-4C5 and the immunoreactive bands identified

Table 4									
Inhibition of Binding of IL-1 Proteins to Different Subtypes and Forms of the Murine IL-1 Receptor by Anti-Receptor Antibodies									
IL-1s	Type I				Type II				
	Natural	Recombinant		Soluble ⁷	Natural	70Z/3 ⁵	Recombinant	COS ⁸	
		EL-4 ⁴	CHO ⁶						
<u>Inhibition by 35F5 (anti-Type I)</u>									
Mu IL-1 α	+ ¹		+		-			-	
Mu IL-1 β	+		+		-	ND		ND	
Hu IL-1 α	+		+		-	+		-	
Hu IL-1 β	+		+		-	+		ND	
Hu IL-ra	50%		ND		-	+		ND	
<u>Inhibition by 4C5 (anti-Accessory Protein)</u>									
Mu IL-1 α	-		-		+			-	
Mu IL-1 β	+		-		+	ND		ND	
Hu IL-1 α	-		-		+	-		-	
Hu IL-1 β	+		-		+	ND		-	
Hu IL-1ra	-		ND		ND	ND		ND	
<u>Inhibition by 4E2 (anti-Type II)</u>									
Mu IL-1 α	-		ND		+	ND		+	
Mu IL-1 β	-		ND		+	ND		ND	
Hu IL-1 α	-		ND		+	ND		+	
Hu IL-1 β	-		ND		+	ND		ND	

1. + and -; Antibody blocks 100% and less than 10%, respectively, of IL-1 binding at 0.1 mg/ml.
2. Mu IL-1 α and Mu IL-1 β = murine IL-1 α and IL-1 β , respectively.
3. Hu IL-1 α , Hu IL-1 β , and Hu IL-1ra = human IL-1 α , IL-1 β , and IL-1ra, respectively.
4. Murine EL-4 cells express approximately 2000 Type I IL-1Rs/cell and an undetectable number of the Type II IL-1R.
5. Murine 7OZ/3 cells express approximately 2000 Type II IL-1Rs/cell and undetectable numbers of Type I IL-1R.
6. Recombinant full length Type I IL-1R expressed in CHO cells.
7. Recombinant Type I IL-1R extracellular domain expressed in a baculovirus system.
8. Recombinant Type II IL-1R expressed in COS cells.

Inhibition of IL-1 Binding to Different IL-1 Receptors by Anti-Receptor Antibodies								
Antibody	IC ₅₀ ($\mu\text{g/ml}$)							
	Type I				Type II			
	Natural ¹		Recombinant ²		Natural ¹		Recombinant ²	
					7OZ/3			
	HuIL-1 α ³	HuIL-1 β ⁴	HuIL-1 α	HuIL-1 β	HuIL-1 α	HuIL-1 β	HuIL-1 α	HuIL-1 β
35F5 (anti-Type I)	.0001	.0015	<.1	<.1	>100	>100	ND	ND
4E2 (anti-Type II)	>100	>100	>100	>100	.1	.25	2	ND
4C5 (anti-accessory protein)	>100	.13	>100	>100	.32	.34	>100	>100

1. Source of natural Type I (EL-4 cells) and Type II (7OZ/3 cells) IL-1Rs used in the inhibition assays.

2. Recombinant Type I or Type II IL-1Rs were expressed in either CHO or COS cells.

3. [¹²⁵I]-Hu IL-1 α as ligand in the assay.

4. [¹²⁵I]-Hu IL-1 β as ligand in the assay.

1. Source of natural Type I (EL-4 cells) and Type II (7OZ/3 cells) IL-1Rs used in the inhibition assays.

2. Recombinant Type I or Type II IL-1Rs were expressed in either CHO or COS cells.

3. [^{125}I]-Hu IL-1 α as ligand in the assay.

4. [^{125}I]-Hu IL-1 β as ligand in the assay.

by autoradiography. A major protein of ~90 kDa and a minor protein of 55 kDa were immunoreactive with radiolabeled 4C5. These two proteins were also identified on the immunoblot if the EL-4 extract was directly purified on a 4C5 affinity matrix. These data indicated
5 that the apparent molecular weight of the natural, glycosylated IL-1R AcP is ~90 kDa and that proteolytic processing may reduce its size to ~55 kDa.

Example 5

10

Neutralization of IL-1 β Biologic Activity by Monoclonal Antibody 4C5

The ability of mAb 4C5 to neutralize IL-1 β biologic activity in a dose-dependent manner was demonstrated in three biologic assays: 1)
15 IL-1 induced proliferation of murine splenic B cells, 2) IL-1 induced proliferation of D10.G4.1 helper T cells, and 3) IL-1 induced kappa light chain expression in 70Z/3 cells. MAb 4C5 demonstrated a dose-dependent inhibition of IL-1 β , but not IL-1 α , induced proliferation of the splenic B cells (Fig. 6). In contrast to mAb 4C5, the anti-Type I
20 receptor antibody 35F5 blocked both IL-1 α and IL-1 β induced proliferation of B cells. The anti-Type II IL-1R antibody 4E2 did not inhibit proliferation induced by either IL-1 α or IL-1 β . In a similar fashion, mAb 4C5 inhibited IL-1 α , but not IL-1 β , induced proliferation of D10.G4.1 T cells (Fig. 7). Both mAb 35F5 and human IL-1ra blocked
25 IL-1 α and IL-1 β induced proliferation of the D10.G4.1 cells. MAb 4C5 also blocked IL-1 β , but not IL-1 α , induced expression of kappa light chain on 70Z/3 cells (Fig. 8). Antibody 35F5 blocked both IL-1 α and IL-1 β induced effects in this assay, whereas mAb 4E2, which recognizes the Type II IL-1R, was inactive. For these assays,
30 neutralization of IL-1 activity by the antibodies or by IL-1ra is detected as a dose-dependent decrease in the biological response. The block in response may be 100% inhibition (i.e. equal to no IL-1 added) or to a lower level depending on the potency of the antibody.

Example 6

Inhibition of IL-1 β Biologic Activity *In Vivo* by Monoclonal Antibody 4C5

5

Mice administered IL-1 show a rapid and dramatic increase in the concentration of IL-6 in their serum. The magnitude of the increase in serum IL-6 is dependent on the IL-1 dose and can be blocked by factors that interfere with IL-1 binding to Type I IL-1R. When tested in this IL-1 biological model, 4C5 blocked by approximately 90% the IL-1 β , but not IL-1 α , induced increase in serum IL-6 (Fig. 9). The anti-Type I IL-1R antibody 35F5 blocked both IL-1 α and IL-1 β induced increase in serum IL-6. A control mAb X-7B2 had no inhibitory effect.

15

Example 7

Expression cloning of Mouse (Murine) IL-1R AcP using Mab 4C5

20 Extraction of RNA

3T3-L1 cells were harvested and total RNA was extracted using guanidinium isothiocyanate/phenol as described (P. Chomczynski and N. Sacchi, Anal. Biochem. 162:156, 1987). Poly A⁺ RNA was isolated from total RNA by one batch adsorption to oligo dT latex beads as described (K. Kuribayashi *et al.*, Nucl. Acids Res. Symposium Series 19: 61, 1988). The mass yield of poly A⁺ RNA from this purification was approximately 6%. The integrity of the RNA preparations was analyzed by fractionating in 1.0% agarose gels under denaturing conditions in the presence of 2.2M formaldehyde (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, 1989).

30

3T3-L1 cDNA library construction

From the above poly A⁺ RNA, a cDNA library was established in the mammalian expression vector pEF-BOS (Mizushima and Nagata, Nucl. Acids Res. 18: 5322, 1990). 10 μ g of poly A⁺ RNA were reverse transcribed using RNaseH⁻ reverse transcriptase (GIBCO BRL Life Technologies Inc., Gaithersburg, MD). The resulting mRNA-cDNA

35

- hybrids were converted into blunt ended doublestranded cDNAs by established procedures (Gubler and Chua, in: Essential Molecular Biology, Volume II, T.A. Brown, editor, pp. 39-56, IRL Press 1991). *Bst*XI linkers (Aruffo and Seed, Proc. Natl. Acad. Sci (USA) 84:8573, 1987) were ligated to the resulting cDNAs and molecules >1000 base pairs (bp) were selected by passage over a Sephacryl SF500 column. The Sephacryl SF500 column (0.8 x 29 cm) was packed by gravity in 10mM Tris-HCl pH 7.8/1mM EDTA/100mM NaAcetate. *Bst*XI linker-treated cDNA was applied to the column and 0.5 ml fractions were collected. A small aliquot of each fraction was fractionated in a 1.0% agarose gel. The gel was dried down by vacuum and the size distribution of the radioactive cDNA was visualized by exposure of the gel to X-ray film. Fractions containing cDNA molecules >1000 bp were selected and pooled. The cDNA was concentrated by ethanol precipitation and ligated to the cloning vector. The cloning vector was the plasmid pEF-BOS that had been digested with *Bst*XI restriction enzyme and purified over two consecutive agarose gels. 375 ng of plasmid DNA were ligated to 18.75 ng of size selected cDNA from above in 150 µl of ligation buffer (50 mM Tris-HCl pH 7.8/10mM MgCl₂/10mM DTT/1 mM rATP/25 mg/ml bovine serum albumin) at 15°C overnight. The following day the ligation reaction was extracted with phenol/chloroform/isoamyl alcohol (25:24:1). The nucleic acids were ethanol precipitated in the presence of 5 µg of oyster glycogen. The precipitate was dissolved in water and ethanol precipitated again, followed by washing with 70% ethanol. The final pellet was dissolved in 14 µl of water and 1 µl aliquots were electroporated into *E. coli* strain DH-10B (GIBCO-BRL). By this method, a library of approximately 4×10^6 recombinants was generated.
- 30 Screening for murine IL-1 Receptor Accessory Protein (muIL-1R AcP) cDNAs by panning with monoclonal antibody 4C5

- The panning method has been described previously (Aruffo and Seed, Proc. Natl. Acad. Sci. (USA) 84: 8573, 1987). Ten aliquots from the 3T3-LI library each representing approximately 5×10^4 clones were plated on LB agar plates containing 100 µg/ml ampicillin (amp) and grown overnight at 37°C. The next day, the colonies from each pool were scraped from the plates into separate 50 ml aliquots of LB +

amp and cultures grown at 37°C for another 2-3 hrs. Plasmid DNA was subsequently extracted using QIAGEN plasmid kits (Qiagen Inc., Chatsworth, CA). The ten separate DNA pools were then used to transfect COS-7 cells by the DEAE dextran technique (5 µg DNA/2x10⁶ cells/9 cm diameter dish) (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press 1989). 72 hrs after transfection, the COS cells were detached from the plates using 0.5 mM EDTA/0.02% Na-azide in phosphate buffered saline (PBS). A single cell suspension was made of each pool. The anti-muIL-1R AcP mAb 4C5 was bound to the cells for 1 hr on ice [(10 µg/ml 4C5 mAb in 3 ml PBS/0.5 mM EDTA/0.02% Na azide/ 5.0% Fetal Calf Serum (FCS)]. The 3 ml of cell-mAb suspension was centrifuged through 6 ml of 2% Ficoll in the above buffer (~300 x g, 5 minutes) to remove unbound mAb. The cells were gently resuspended in the above buffer. The cells from each pool were subsequently added to a single bacterial plate (9 cm diameter) that had been coated with polyclonal goat anti-rat IgG (20 µg/ml in 50 mM Tris-HCl pH 9.5, room temperature, 1.5 hrs) and blocked overnight with PBS/1% BSA at room temperature. COS cells were left on the bacterial plates for 2-3 hrs at room temperature with gentle rocking. Nonadherent cells were gently removed by washing with PBS. The remaining cells were lysed by the addition of 0.8 ml of Hirt lysis solution (0.6% SDS/10 mM EDTA). The lysates were transferred to 1.5 ml Eppendorf tubes and made 1 M NaCl, incubated overnight on ice and spun at 15,000 xg for 15 min at 4°C. The supernatants were extracted with phenol/chloroform/isoamyl alcohol (25:24:1) one time, 10 µg of oyster glycogen was added and the DNA precipitated twice by addition of 0.5 volumes of 7.5 M NH₄OAc and 2.5 volumes of ethanol. The pellet was washed with 70% ethanol, dried and resuspended in 1 µl of H₂O. Each panned pool of DNA was then electroporated into *E. coli* strain DH-10B. After electroporation, 5x10⁴ colonies of each pool were grown as above and plasmid DNA was isolated as above. This DNA represents one round of panning enrichment of the library. A total of three panning rounds were completed keeping each of the ten library pools separate throughout.

After the third round of panning, each of the ten pools was used to transfect COS cells by the DEAE dextran method (1 µg DNA/2x10⁵

cells/well of a 6-well Costar dish). 72 hrs post transfection, the COS cells were screened for pools that expressed muIL-1R AcP by rosetting with secondary antibody coated polystyrene beads (DynaI Inc., Great Neck, NY). 4C5 mAb was bound to transfected COS cells in
5 PBS/2% FCS (2 μ g Ab/well) for 1.5 hrs at room temperature with gentle rocking. Antibody was removed and cells were washed with PBS/2% FCS. 1 ml PBS/2% FCS/1 μ l of sheep anti-rat IgG coated polystyrene beads ($\sim 4 \times 10^5$ Dynabeads M-450) was added and incubated 1.5 hrs at room temperature with gentle rocking. The beads
10 were removed and the cells washed 5-10 times with PBS. Cells were then fixed by incubation in 95% ethanol/5% acetic acid and examined microscopically for rosetting. One of the ten pools (panning pool #2) was found positive for surface expression of muIL-1R AcP.

15 To identify positive clone(s), 100 μ l of LB + amp was placed in the wells of two 96-well microtiter plates. Each well was then inoculated with 4 individual colonies from panning pool #2. The bacterial cells were allowed to grow for 5-6 hrs at 37°C. Pools were then made by combining 10 μ l aliquots from each well in the 8 rows and 12 columns
20 of each plate, keeping each row and column separate. These pools were each used to inoculate a separate 5 ml culture in LB + amp and grown overnight at 37°C. The next day plasmid DNA was isolated using QIAGEN plasmid kits. Each DNA preparation represented pools of either 48 (rows) or 32 (columns) individual isolates from panning
25 pool #2. Each microtiter pool was used to transfect COS cells in 6-well plates as above and 72 hrs after transfection the cells were screened for Dynabead rosetting as above. Two positive pools were found from one of the microtiter plates, one from row E and one from column 2. A 10 ml aliquot was taken from the well at the intersection of the
30 column and row (well E2) and plated onto LB agar + amp. After overnight incubation, 40 individual colonies were used to each inoculate a 5 ml LB + amp culture. Plasmid DNA was isolated from these cultures using QIAGEN plasmid kits. Each plasmid isolate was digested with *Xba*I restriction enzyme, to release the cDNA insert, and
35 fractionated on a 1.0% agarose gel. This analysis revealed that only three sizes of cDNA inserts were represented in the positive microtiter pool. A single representative of each of the three plasmids was used to transfect COS cells in a 6-well plate as above and screened by

rosetting with Dynabeads. In this way a single cDNA clone (E2-K) was identified that encoded the 4C5-reactive muIL-1R AcP.

Characterization of muIL-1R AcP cDNA's

5

The cDNA clone E2-K (pEF-BOS/muIL-1R AcP) was initially characterized by restriction enzyme mapping. Digestion of this clone with *Xba*I released a 3.2 kilobasepair (kb) cDNA insert. The 3.2 kb *Xba*I fragment was gel-purified and the DNA sequence of both strands
10 was determined by using an ABI automated DNA sequencer along with thermostable DNA polymerase and dye-labeled dideoxy-nucleotides as terminators. The DNA sequence revealed an open reading frame (ORF) in the 5-prime half of the clone (see below). Restriction enzyme mapping using Intelligenetics computer software
15 indicated a 1.4 kb *Pst*I restriction fragment within the ORF. This 1.4 kb fragment was gel isolated and used as a probe to identify additional muIL-1R AcP cDNA clones. Approximately 6×10^5 additional clones from the 3T3-L1 cDNA library described previously were plated as above. Colony lifts were performed (Molecular Cloning,
20 A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press 1989) and the lifts were probed with the 1.4 kb *Pst*I restriction fragment labelled with [³²P]-dCTP by random-priming using the Multiprime DNA labelling system (Amersham Co., Arlington Heights, IL). In this way two
25 additional homologous cDNA clones were isolated. One contained a 1.0 kb insert and the other a 4.3 kb insert as determined by *Xba*I digestion. The DNA sequence of the 4.3 kb insert was determined as above to confirm the sequence of the muIL-1R AcP ORF.

30 Sequence analysis of muIL-1R AcP cDNA clone

The nucleotide sequence of the open reading frame in the muIL-1R AcP cDNA insert is shown in Figure 10A. [SEQ ID NO:4] This open
reading frame (ORF) consists of 1710 bp, which encodes a protein of
35 570 amino acids. The amino acid sequence, shown in Figure 10B [SEQ ID NO:6], predicts a 20 amino acid NH₂-terminal signal peptide with cleavage after Ala-1, an extracellular domain from Ser1-Glu339, a hydrophobic transmembrane domain from Leu340-Leu363 and a

cytoplasmic tail from Glu364 to the COOH-terminus. Seven potential N-linked glycosylation sites are all contained within the extracellular domain.

5 Database searches with the protein sequence using the
Intelligenetics computer program indicate that muIL-1R AcP has
significant homology to both IL-1 Type I and IL-1 Type II receptors
from mouse, human, chicken and rat. The homology to each of these
proteins is approximately 25% and is uniformly distributed
10 throughout the protein sequence. Further analysis of the amino acid
sequence of muIL-1R AcP shows it to be a member of the
immunoglobulin superfamily. The three pairs of cysteine residues,
conserved in the extracellular domain of all of the IL-1 receptors and
responsible for formation of three IgG-like domains, are perfectly
15 conserved in muIL-1R AcP.

Example 8

Mab 4C5 binding to Murine Recombinant IL-1R AcP Expressed in COS
20 cells

To confirm that the cDNA for muIL-1R AcP encodes a protein
reactive with mAb 4C5, recombinant muIL-1R AcP was expressed on
transfected COS cells and examined for direct binding of [125 I]-4C5.
25 COS cells were electroporated, by standard methods, with pEF-
BOS/muIL-1R AcP. After electroporation, cells were seeded onto a 6
well tissue culture plate at $2-3 \times 10^5$ cells/well. After 48-72 hrs
growth medium was removed and 1 ml of binding buffer
(RPMI/5%FCS) containing 1×10^6 cpm of [125 I]-4C5 was added per
30 well either alone (total binding) or in the presence of 2 μ g unlabelled
4C5 as cold inhibitor (non-specific binding). Both total and non-
specific binding were carried out in duplicate. After 3 hrs incubation
at 4 $^{\circ}$ C, binding buffer was removed and the cells were washed 3
times with PBS. The cells were then lysed by addition of 0.75 ml of
35 0.5% SDS. The lysates were harvested and bound counts were
determined. Specific binding was calculated by subtracting non-
specific counts from total counts. Specific counts were approximately
30,000 cpm/ well with a non-specific background of 8% indicating

that pEF-BOS/muIL-1R AcP directs the expression of 4C5 immunoreactive protein in COS cells.

The size of recombinant muIL-1R AcP expressed in COS cells was
5 determined by metabolic labelling of transfected COS cells with [³⁵S]-
methionine and immunoprecipitation of labelled muIL-1R AcP with
the mAbs 4C5 or 2E6 (Table 2). 36 hrs after electroporation with pEF-
BOS/muIL-1R AcP, medium was removed and COS cells were washed
1 time with methionine-free medium [DMEM(high glucose, without
10 methionine-GIBCO-BRL)/10% FBS/1 mM L-glutamine/ 1 mM Na
pyruvate)]. Fresh methionine-free medium was added and after 5-8
hrs incubation at 37° C, 50-100 µCi of ³⁵S-methionine was added per
ml of medium and incubation continued for 24 hrs. Medium was then
removed and the cells washed 2 times with cold PBS. Cells were
15 solubilized by the addition of RIPA buffer (0.5% NP-40, 0.5% Tween-
20, 0.5% Deoxycholate, 420mM NaCl, 10mM KCl, 20mM Tris pH 7.5,
1mM EDTA) and incubation on ice for 15 min. The lysate was
transferred to tubes and spun at 15,000 x g for 15 min. Lysates were
precleared by the addition of 40 µl of GammaBind G Sepharose (50%
20 v/v in RIPA buffer) (Pharmacia Biotech Inc., Piscataway, NJ) to 500 µl
of lysate and incubation overnight at 4° C. The next day the
precleared lysates were spun 30 sec in a microfuge and lysates were
transferred to clean tubes. Another 40 µl of GammaBind G Sepharose
was added along with 20 µg mAb 4C5 or 2E6 (Table 2) and the
25 immunoprecipitations were incubated for 3 hrs at 4° C with rotation.
The Sepharose-Ab complexes were spun down and washed 1X with
RIPA buffer, 1X with 50mM HEPES pH 7.9/200mM NaCl/1mM
EDTA/0.5% NP-40 and 1X with 25mM Tris pH 7.5/100mM NaCl/0.5%
Deoxycholate/1.0% Triton X-100/0.1% SDS. Protein was released from
30 the beads by addition of 20 µl of 2X Laemmli sample buffer (Laemmli,
Nature 227:680, 1970). The proteins were separated by electro-
phoresis in Tris-Glycine PAGE and visualized by autoradiography. As
shown in Figure 11, recombinant muIL-1R AcP immunoprecipitated
with mAb 4C5 or 2E6 from transfected COS cells migrates as a broad
35 band from 70-90 kDa. No protein was precipitated from mock
transfected COS cells.

Example 9

Expression of Recombinant IL-1R AcP in COS Cells: Reactivity with [125 I]-Labeled IL-1 Proteins and Monoclonal Antibodies

5

The binding characteristics of the recombinant IL-1R AcP for [125 I]-labeled IL-1, 4C5 and 4E2 were determined (Fig. 12). The data showed high level expression of recombinant IL-1R AcP [Cos(4C5)] as determined by [125 I]-4C5 binding, but no increase in [125 I]-human
10 IL-1 α binding when compared to control transfected COS cells [Cos(PEF-BOS)]. For comparison, the high level expression of murine recombinant Type I receptor in COS cells [COS (Mu-IL-1R)] as determined by [125 I]-35F5 binding was accompanied by a corresponding increase in radiolabeled human IL-1 β and IL-1 α
15 binding (Fig. 13).

Example 10

Purification of Natural Murine IL-1 Receptor Accessory Protein (IL-1R 20 AcP) from EL-4 Cells

Murine EL-4 cells (100 gm) were solubilized in 1 liter of PBS containing 8 mM CHAPS, 5 mM EDTA and the protease inhibitors pepstatin (10 μ g/ml), leupeptin (10 μ g/ml), benzamidine (1 mM),
25 aprotinin (1 μ g/ml) and PMSF (0.2 mM). After centrifugation at 100,000 x g to remove insoluble material, the supernatant was loaded onto a 50 ml wheat germ agglutinin (WGA) agarose column (Vector Laboratories, Inc.) at 0.8 ml/min. The column was washed with equilibration buffer (PBS, 8 mM CHAPS, 5 mM EDTA) followed by
30 equilibration buffer containing 0.5 M NaCl, and bound protein was eluted with PBS containing 8 mM CHAPS and 0.3 M N-acetyl-D-glucosamine.

The sugar-eluted fractions from three WGA agarose column runs
35 were pooled and loaded onto a 5 ml immunoaffinity column [mAb 4C5 antibody cross-linked to Protein G Sepharose via dimethylpimelimidate (Stern, A.S. and Podlaski, F.J., in: Techniques in Protein Chemistry IV, R.H. Angeletti, ed., pp. 353-360, Academic Press, NY,

1993)] equilibrated with PBS containing 8 mM CHAPS at 1 ml/min. The column was washed with equilibration buffer followed by equilibration buffer containing 1 M NaCl. Bound protein was eluted with 50 mM diethylamine buffer, pH 11.5, containing 8 mM CHAPS.

5 The fractions containing IL-1R AcP were dialyzed against PBS containing 4 mM CHAPS and concentrated.

All column fractions were monitored for the presence of IL-1R AcP by SDS-PAGE/immunoblot analysis with mAb 4C5. SDS-PAGE was

10 performed on 8-16% gradient gels (Novex), and proteins were transferred to nitrocellulose as described (Towbin et al., Proc. Natl. Acad. Sci. USA 76: 4350, 1979). After blocking the nitrocellulose with 2.5% casein in 50 mM Tris containing 150 mM NaCl₂ and 0.01% thimerosal (pH 7.5), blots were incubated with mAb 4C5 (5 µg/ml)

15 followed by incubation with HRP-conjugated goat (Fab)₂ anti-rat antibody (Tago Immunologicals). Blots were developed with the ECL System (Amersham Life Science).

The amino acid composition (Hollfelder et al., J. Protein Chem.

20 12: 435, 1993) of the final protein preparation is shown in Table 6; it is similar to the composition predicted from the deduced protein sequence [SEQ ID NO: 3] from the cDNA clone [SEQ ID NO:1] (Figure 16). The remainder of the sample was subjected to SDS-PAGE, transferred to a PVDF membrane (Matsudaira, J. Biol. Chem. 262: 10035, 1987)

25 and stained with Coomassie blue R-250. The protein-stained band at 80 kDa, which was immunoreactive with 4C5 antibody, was analyzed by NH₂-terminal sequence analysis (Hollfelder et al., J. Protein Chem. 12: 435, 1993). Two sequences were obtained (1-3 pmoles of each amino acid per cycle), one of which matched residues 1-12

30 (SERXDDWXLDTM) of the deduced protein sequence obtained from expression cloning of murine IL-1R AcP (Figure 10B).

Although IL-1R AcP solubilized from EL-4 cells has a M_r = 80 kDa as determined by immunoblot analysis with the 4C5 antibody, the

35 predicted molecular weight of the protein from the cDNA sequence is 66 kDa. This apparent difference is likely due to glycosylation of the accessory protein. To address this issue, the affinity purified IL-1R AcP was subjected to SDS-PAGE, and the Coomassie blue-stained band

corresponding to the 80 kDa, 4C5-immunoreactive protein was eluted from the gel and chemically deglycosylated with trifluoromethane sulfonic acid (Edge et al., Anal. Biochem. 118: 131, 1981). The deglycosylated protein migrates with a $M_r = 63-64$ kDa in SDS-PAGE, a value in good agreement with the predicted molecular weight from the cDNA sequence.

Example 11

10 Isolation of Genomic Clones of Human IL-1 Receptor Accessory Protein

Screening by cross-hybridization

Attempts were made to identify and isolate a cDNA coding for the human homologue of IL-1R AcP by screening human cDNA libraries by

Table 6	
Amino Acid Composition of Natural Murine IL-1 Receptor Accessory Protein from EL-4 Cells	
amino acid	mole %
Cys	n.d.
Asx	10.5
Thr	5.3
Ser	5.1
Glx	13.1
Pro	n.d.
Gly	8.5
Ala	8.9
Val	7.4
Met	2.7
Ile	6.9
Leu	10.6
Thr	3.3
Phe	4.5
His	2.1
Lys	5.7
Trp	n.d.
Arg	5.4
n.d. = not determined	

20 cross-hybridization with sequences from murine IL-1R AcP. Human cDNA libraries prepared from mRNA isolated from RAJ1 cells or NC37 cells were probed with the murine IL-1R AcP cDNA, but initial

attempts were unsuccessful, possibly due to very low expression of the human homologue in these cells (see Example 12). We decided to screen a human genomic library to isolate specific sequences that could be used to subsequently screen a human cDNA library.

5

The murine IL-1R AcP cDNA clone [3.2 kb *Xba*I fragment] and restriction fragments of the murine IL-1R AcP cDNA clone [1.4 kb *Pst*I fragment and 843 basepair (bp) *Bam* HI/*Sal*I fragment] were used as probes to perform low-stringency Southern blot analysis of human
10 genomic DNA (Clontech, Palo Alto, CA). This analysis was performed to determine optimal hybridization and washing conditions under which the murine probe could detect homologous sequences present in the human genome. Hybridization with the murine IL-1R AcP-cDNA probes were carried out at 37°C overnight in hybridization buffer A
15 (2X SSC, 20% formamide, 2X Denhardt's, 100 µg/ml yeast RNA, 0.1% SDS). Probes were labelled with [³²P]-dCTP using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). The blots were washed with 2X SSC and 0.01% SDS at various temperature points beginning at 37°C. The optimal conditions were determined to be the
20 use of the [³²P]-843 bp *Bam*HI/*Sal*I fragment, hybridizing at 37°C overnight in hybridization buffer A, washing in 2X SSC, 0.01% SDS at 55°C. These conditions yielded the lowest background and were used to screen a commercially available human genomic library.

25 To identify human genomic clones of IL-1R AcP, a human lung fibroblast library in Lambda FIX #944201 (Stratagene, La Jolla, CA) was screened. 4.8×10^5 plaques were screened by standard plaque hybridization techniques (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring
30 Harbor Laboratory Press, 1989) using the conditions described above. Six hybridization positive phage clones were purified by successive plaque hybridization. Two phage clones were further characterized (#1 and #7).

35 Characterization of human genomic clones

The human IL-1R AcP genomic clones were initially characterized by restriction enzyme mapping. Bacteriophage lambda

DNA was isolated from clones #1 and #7 using LambdaSorb phage adsorbent (Promega, Madison, WI). The phage DNAs were digested with *SacI* to release the inserts, and the fragments were then separated by electrophoresis on 1% agarose gels. Inserts for both clones #1 and #7 were ~17 kb in size. Further mapping of clones #1 and #7 was performed using *XbaI* and *EcoRI*. The digested DNAs were separated by electrophoresis on 1% agarose, transferred to a nylon membrane (ICN, Irvine, CA) and crosslinked for Southern blot analysis. The membrane was hybridized with the 843 bp (*BamHI/SalI*) fragment of murine IL-1R AcP previously described. The probe was labelled with [³²P]-dCTP using Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). The blots were hybridized and washed using the low stringency hybridization conditions previously described.

15

A 4.5 kb fragment from the *EcoRI* digest and a 2.6 kb fragment from the *XbaI* digest were identified as positive for hybridization to the murine IL-1R AcP sequences. The 4.5 kb fragment and the 2.6 kb fragment were isolated from 0.8% Seaplaque agarose (FMC, Rockland, ME) and purified with Qiaex (Qiagen, Chatsworth, CA). The fragments were subcloned into the vector pBluescript II SK⁺ (Stratagene, La Jolla, CA) to facilitate characterization. Plasmid DNA was prepared using the Qiagen plasmid kit (Qiagen, Chatsworth, CA).

Southern blot analysis was performed to determine which fragment would be more suitable to detect homologous sequences in the human genome. The 4.5 kb and 2.6 kb fragments were used as probes. Low stringency hybridization conditions were used as follows: 5X SSC, 50% formamide, 5X Denhardt's, 100 µg/ml yeast RNA, 0.1% SDS, 37°C, overnight hybridization. Probes were labelled with [³²P]-dCTP using Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). The membranes were washed using high stringency conditions (0.1 X SSC, 0.01% SDS) at various temperature points beginning at 37°C. Optimal conditions were determined to insure selecting a probe that would be specific for huIL-1R AcP when screening a human cDNA library. These optimal conditions are described in Example 12.

35

Sequence analysis of human genomic clone

The pBluescript II SK⁺/2.6 kb human genomic IL-1R AcP plasmid DNA was sequenced using an ABI automated DNA sequencer
5 along with thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators. Preliminary DNA sequence analysis showed that this DNA contained a 150-nucleotide region with 90% homology to a sequence coding for the intracellular domain of the murine IL-1R AcP.

10

Example 12

Isolation of cDNA Clones of Human IL-1R AcP

15 YT cell cDNA library construction

The mAb 2E6 (Example 2, Table 2) was originally characterized by its reactivity with the murine IL-1R AcP. Preliminary data indicated that mAb 2E6 detects the IL-1R AcP on human cells. A
20 number of human cell lines were screened with [¹²⁵I]-2E6 and it was determined that the YT cell line (Yodoi et al., J. Immunol. 134: 1623, 1985) expressed relatively high numbers of 2E6 reactive sites per cell compared to other human cell lines, e.g. RAJI. The YT cell line was therefore chosen as the source of RNA for cDNA library construction.

25

Total RNA was extracted from YT cells and cDNA was made from this RNA as described herein (Example 7: 3T3-LI cDNA library construction). *Eco*RI adapters (Stratagene, La Jolla, CA) were ligated to the resulting cDNAs and molecules >1000 bp were selected by passage
30 over a Sephacryl SF500 column as described herein (EXAMPLE 7: 3T3-LI cDNA library construction). The cDNA was concentrated by ethanol precipitation and ligated to the cloning vector. The cloning vector was Lambda ZAP II phage (Stratagene) that had been digested with *Eco*RI restriction enzyme and dephosphorylated (as provided by the
35 supplier). 10 aliquots of 100ng of size selected cDNA from above were each ligated to 1 µg of Lambda ZAP II arms (*Eco*RI digested and dephosphorylated) in 5 µl of ligation buffer (66 mM Tris-HCl pH 7.5/5mM MgCl₂/1mM DTE/1mM rATP) at 15°C overnight. The

following day the ligations were pooled and packaged into Lambda phage in twelve 4- μ l aliquots using Gigapack II packaging extracts and following the manufacturer's instructions (Stratagene). Packaged phage were titered by plating in bacterial strain XL1-Blue-MRF' (Stratagene) in the presence of 5 mM Isopropyl- β -D-thiogalactopyranoside (IPTG) (Boehringer Mannheim Co., Indianapolis, IN) and 4 mg/ml 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal) (Boehringer-Mannheim) to distinguish non-recombinant phage. Plaque counts the following day indicated that a library of 3.55×10^6 recombinants was obtained with a non-recombinant background of <0.1%.

Screening of human cDNA library by hybridization with human genomic clone fragments of IL-1R AcP

15

The 2.6 kb *Xba*I restriction fragment which was previously described as being a specific probe for the huIL-1R AcP was used at low stringency hybridization (5X SSC, 50% formamide, 5X Denhardt's, 100 μ g/ml yeast RNA, 0.1% SDS, 37°C overnight), high stringency wash conditions (0.1X SSC, 0.01% SDS, 40°C) to screen the YT cDNA library. 4.8×10^5 plaques were screened by standard plaque hybridization techniques (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.I. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, 1989). Three hybridization positive phage clones (#3, #5, and #6) were identified and purified by successive plaque hybridization. Excision of pBluescript SK (-) phagemids containing insert DNA from the Lambda Zap II vector was performed according to manufacturer's protocol.

30 Characterization of human cDNA clones

The human IL-1R AcP cDNA inserts #3, #5, and #6 in pBluescript SK (-) were further characterized by restriction enzyme mapping. Initially, miniprep plasmid DNA was prepared by the rapid boil method (Holmes and Quigley, Anal. Biochem. 114: 193, 1981). Subsequently, plasmid DNA was prepared with the Qiagen plasmid kit. The plasmid DNAs were digested with *Eco*RI to release the inserts, and the inserts were separated by electrophoresis on 1% agarose. Clone #3

contained a 2.3 kb insert, clone #5 contained a 1.4 kb insert, and clone #6 contained a 2.7 kb insert. Further restriction mapping indicates a single *PvuII* site present in all three clones.

5 Sequence analysis of human IL-1R AcP cDNA clones

Plasmid DNA from clones #3, #5 and #6 were sequenced using an ABI automated DNA sequencer along with thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators.

- 10 Preliminary sequence analysis indicated that only clones #3 and #6 had inserts that were homologous to the murine IL-1R AcP cDNA. Therefore, clones #3 and #6 inserts were sequenced completely. Sequence analysis indicates that clones #3 and #6 are overlapping clones. Schematic representations of clones #3 and #6 are shown in
- 15 Figure 14. Clone #3 contains the ATG initiation codon and the 5' portion of the coding region. Clone #6 contains the 3' portion of the cDNA and the TGA stop codon. These two overlapping clones were used to construct a full length huIL-1R AcP cDNA.

20

Example 13

Construction of Full Length Human IL-1R AcP cDNA

- Restriction endonuclease mapping and preliminary sequence analysis indicated that there was a single *BstXI* site present in clone
- 25 #3 and clone #6. Shown in Figure 14 is a schematic representation of overlapping clones #3 and #6. Clones #3 and #6 were digested with the restriction enzymes *BstXI* and *XbaI*. Fragments of approximately 846 bp and approximately 2700 bp were prepared from clone #3 and clone #6, respectively, by electrophoresis in 0.7% Seaplaque agarose
- 30 (FMC, Rockland, ME) and purified with Qiaex (Qiagen, Chatsworth, CA).

- The full-length human IL-1R AcP was prepared by subcloning into the mammalian expression vector pEF-BOS (Mizushima and Nagato, Nuc. Acids Res. 18: 5322, 1990). pEF-BOS plasmid DNA was
- 35 digested with *XbaI*, treated with calf intestinal phosphatase (Boehringer Mannheim, Indianapolis, IN), separated by electrophoresis on a 0.7% Seaplaque agarose gel, and purified with Qiaex (Qiagen, Chatsworth, CA). The 846 bp and approximately 2700 bp

*Bst*XI/*Xba*I fragments described above were ligated into the *Xba*I-cleaved pEF-BOS expression vector, and the ligation products were transformed into MC1061 competent cells. The transformed cells were plated onto LB agar plates containing 100 µg/ml ampicillin and grown overnight at 37°C. The next day, 12 individual colonies were picked, inoculated into LB and ampicillin (100 µg/ml) and incubated overnight at 37°C. Miniprep plasmid DNA was prepared from each inoculated colony by the rapid boil method (Holmes and Quigley, Anal. Biochem. 114: 193, 1981). Restriction endonuclease analysis confirmed that 10 clones contained the appropriate insert in the proper orientation relative to the promoter region in pEF-BOS.

Plasmid DNA was isolated from two positive clones #1 and #9 by the Qiagen method (Qiagen, Chatsworth, CA). The nucleotide sequence of both strands of both plasmids was determined as described in Example 7. The sequence of the 1710 bp open reading frame (ORF) contained within the full-length huIL-1R AcP cDNA is shown in Figure 15. [SEQ ID NO:1] The deduced amino acid sequence, shown in Figure 16 [SEQ ID NO:3], would encode a protein of 570 residues consisting of a 20 amino acid signal peptide (Met²⁰-Ala⁻¹), a putative extracellular domain (Ser1-Glu339), a hydrophobic transmembrane domain (Leu340-Leu363), and a cytoplasmic tail (Glu364-Val550). Seven potential N-linked glycosylation sites are all contained within the extracellular domain. All seven sites are conserved between murine and human IL-1R AcP.

Example 14

Expression of Soluble Human IL-1R AcP

30

To express the huIL-1R AcP, a soluble form of the protein was engineered for expression in the baculoviral expression system. This system is useful for overproducing recombinant proteins in eukaryotic cells (Luckow and Summers, Bio/Technology 6: 47, 1988). Using the polymerase chain reaction (PCR) method (Innis M.A., et al., PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego), an amplicon was produced that encoded a soluble form of the extracellular domain of huIL-1R AcP. Briefly, two oligonucleotide

primers were synthesized on an Applied Biosystems synthesizer. The forward primer contained the *Bam*HI site and the codons for the first 11 amino acids of the signal peptide: (5')GGCC GGA TCC ATG ACA CTT CTG TGG TGT GTA GTG AGT CTC TAC (3') [SEQ ID NO:10]. The reverse primer sequence coded for the 11 amino acids just before the transmembrane domain, an Ala spacer, and a Glu-Glu-Phe tag, followed by the termination codon TAG and a *Kpn*I site: (5') CGCGCG GGT ACC CTA GAA CTC TTC AGC TTC CAC TGT GTA TCT TGG AGC TGG CAC TTT CTGC(3') [SEQ ID NO:11]. The Glu-Glu-Phe tripeptide tag at the COOH-terminus was engineered to provide an epitope for antibody detection of the recombinant protein. This tripeptide tag is recognized by a commercially available monoclonal antibody to α -tubulin (Skinner et al., J. Biol. Chem. 266: 14163, 1991).

The forward and reverse primers were used to amplify the extracellular domain of the huIL-1R AcP, using clone #3 (Figure 14) as template. The resulting approximately 800 bp PCR amplicon was digested with *Bam*HI and *Kpn*I. The digested fragment was subjected to electrophoresis through 0.7% Seaplaque agarose and purified with Qiaex (Qiagen, Chatsworth, CA). The soluble human IL-1R AcP extracellular domain was then subcloned into pNR1, a derivative of the baculovirus transfer vector pVL941 (PharMingen, San Diego, CA). pNR1 was prepared from pVL941 by removal of the *Eco*RI site at position 7196 (cleavage with *Eco*RI and filling in of sticky ends with Klenow DNA polymerase). The DNA was then subjected to religation, then cleavage with *Bam*HI and *Asp*718 (*Kpn*I isoschizomer) and insertion of the following oligonucleotides which contain *Bam*HI, *Eco*RI, and *Asp*718 recognition sequences:

(5') GATCCAGAATTCATAATAG (3') [SEQ ID NO:12]
(3') GTCTTAAGTATTATCCATG (5')[SEQ ID NO:13]

The *Bam*HI, *Eco*RI, and *Asp*718 restriction sites are unique in pNR1.

pNR1 plasmid DNA was digested with *Bam*HI and *Kpn*I and purified from a 0.7% Seaplaque agarose gel with Qiaex (Qiagen, Chatsworth, CA). The *Bam* HI/*Kpn*I approximately 800 bp huIL-1R AcP PCR amplicon fragment was ligated into the *Bam*HI/*Kpn*I cleaved pNR1 expression vector. The ligation products were transformed into

MC1061 competent cells, which were then plated onto LB agar containing ampicillin (100 µg/ml) and grown overnight at 37°C. The next day, 36 independent colonies were picked and inoculated into LB and ampicillin (100 µg/ml). Miniprep DNA was prepared by the rapid
5 boil method (Holmes and Quigley, Anal. Biochem. 114: 193, 1981). The DNA was analyzed by restriction endonuclease mapping. Thirty plasmid clones were shown to contain the correct insert. Plasmid DNA was prepared from two positive clones (#11, #25) by the Qiagen method (Qiagen, Chatsworth, CA). These clones were verified by
10 sequence analysis.

The pNR1/soluble human IL-1R AcP DNA (clone #25) was co-transfected with linearized AcRP23.lac Z baculovirus DNA (PharMingen, San Diego, CA) into Sf9 (*Spodoptera frugiperda*) cells
15 using the BaculoGold Transfection Kit (PharMingen, San Diego, CA). Following transfection, recombinant baculovirus were isolated and plaque purified according to a protocol described in the BaculoGold Transfection Kit (PharMingen). Plaques were visualized by staining with MTT as described (Shanafelt, Biotechniques 11: 330, 1991).
20 Twelve individual viral plaques were isolated and the virus particles were eluted from the agarose into 0.5 mls of SF-9 media (IPL-41 + 10% FBS - JRH Biosciences, Lenexa, KS) by incubating overnight at 4°C on a rotator. Each recombinant virus was analyzed for the presence of insert by PCR analysis and for the expression of recombinant human
25 IL-1R AcP by immunoblot analysis. For PCR amplification, viral DNA was extracted, incubated with Taq DNA polymerase and the appropriate pNR1 forward and reverse primers (relative to the *Bam*HI/*Asp*718 cloning sites), and amplified using standard PCR methods (Innis *et al.*, PCR Protocols, Academic Press, San Diego 1990).
30 Each amplicon was analyzed by electrophoresis on 1.5% agarose. The results confirmed that 10 out of the 11 plaques tested contained an insert of ~ 1 kb, corresponding to the proper insert size.

For immunoblot analysis, human IL-1R AcP + tag (from the
35 supernatant of Sf9 cells infected with recombinant virus) was isolated by reacting with biotinylated anti-tubulin antibody (YL1/2) (Harlan Bioproducts) immobilized on streptavidin-agarose (Pierce, Rockford, IL). Proteins were eluted from the anti-tubulin antibody matrix with

0.2M glycine pH 2.7, and the fractions neutralized with 3M Tris base. Eluted proteins were treated with Laemmli sample buffer without β -mercaptoethanol, separated on 8% acrylamide (Novex) slab gel and transferred to 0.2 μ nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The immobilized proteins were probed with the YL1/2 antibody (10 μ g/ml), and peroxidase-conjugated goat-anti-rat antibody (1:10,000 dilution) (Boehringer Mannheim Biochemicals). Immunoreactive bands were visualized by ECL (Amersham) according to the manufacturer's protocol. This analysis identified a protein of >200 kDa, that was expressed by recombinant virus containing the human IL-1R AcP + tag insert.

Recombinant virus from plaques #2 and #12 (identified by immunoblot analysis as expressing human IL-1R AcP + tag)were amplified to obtain virus stocks which were used in the large-scale production of human IL-1R AcP + tag for immunization purposes. Sf9 cells were cultured in logarithmic growth (1×10^6 cells/ml) in EX-CELL 401 with 1% Fetal Bovine Serum (JRH Biosciences, Lenexa, KS) at 27°C, infected with recombinant baculovirus as described (O'Reilly *et al.*, Baculovirus Expression Vectors, a Laboratory Manual, Oxford Univ. Press, 1994) and spent culture media were harvested at 3-5 days post-infection. The cells were removed from the spent culture media by centrifugation and the soluble human IL-1R AcP + tag was purified over an affinity matrix composed of immobilized YL1/2 antibody as described in Example 15 below. The purified human IL-1R AcP + tag was used to immunize mice.

Example 15

Preparation and Screening for Monoclonal Antibodies Specific for Human IL-1 Receptor Accessory Protein (huIL-1R AcP)

Three methods are employed to develop antibodies specific for the huIL-1R AcP.

Immunization of mice and rats with COS cells expressing human recombinant IL-1R AcP

COS cells (4×10^7) are transfected by electroporation with the full-length huIL-1R AcP expression plasmid (20 μ g, described in Example 13) in a BioRad Gene Pulser at 250 μ F and 350 volts as per the manufacturer's protocol. The transfected cells are plated into a 250 mm x 250 mm Nunc tissue culture tray and harvested after 72 hrs growth. The transfected cells are released from the tissue culture tray by treatment with NO-zyme (JRH Biosciences) for 10 min at 37°C. The cells are harvested, washed in PBS, pH 7.4 and used for immunizations. Mice and rats are immunized by the intraperitoneal (i.p.) route with COS cells expressing huIL-1R AcP (1×10^7 cells/animal) on Days 0, 7, 14 and 28. On day 40, the animals are bled to determine the titer of the antibody response against huIL-1R AcP (see below for specific assays). Animals are given booster immunizations (1×10^7 cells, i.p.) at 2-4 week intervals after day 40. Serum antibody titers specific for huIL-1R AcP are determined at 10-12 days after each booster immunization. When the animals develop a sufficient serum antibody titer (e.g., 1/1000 dilution of the serum immunoprecipitates at least 50% of a given amount of the complex of [125 I]-IL-1 β crosslinked to IL-1R AcP solubilized from human YT and RAJI cells), they are given booster immunizations in preparation to isolating their spleen cells. These final booster immunizations are composed of 1×10^7 cells given both i.v. and i.p. on two consecutive days. Three days after the last immunization, spleen cells are isolated from the animal and hybridoma cells are produced as described previously. Hybridoma cells secreting antibodies specific for huIL-1R AcP are identified by the assays described below. Hybridoma cells are cloned as described previously in Example 1.

Immunization of mice and rats with purified human recombinant soluble IL-1R AcP

a. Preparation of human recombinant soluble IL-1R AcP in COS cell and baculovirus expression systems. As described above, COS cells are transfected with plasmid DNA expressing the extracellular domain of huIL-1R AcP that has a tag (Glu, Glu, Phe) (Skinner et al., J. Biol.

Chem. 266: 14163, 1991) inserted at the C-terminus (soluble IL-1R AcP, amino acids 1-339 + Ala + Glu + Glu + Phe). The tag encodes the sequence for recognition by the anti-tubulin antibody YL1/2 (Harlan Bioproducts). The medium is harvested from the cells 72 hrs after
5 transfection and soluble IL-1R AcP+tag is detected and purified as described below.

Standard methods (Gruenwald and Heitz, Baculovirus Expression Vector System: Procedures and Methods Manual, Second Edition, 1993,
10 PharMingen, San Diego, CA) are employed to generate a pure recombinant baculovirus expressing the soluble IL-1R AcP protein. Briefly, plasmid DNA coding for the soluble extracellular domain of human IL-1R AcP+tag is inserted into the transfer vector pNR1 as described in Example 14. The recombinant transfer vector is purified
15 and co-transfected with linearized ACVW1.lacZ DNA (PharMingen) into Sf9 (*Spodoptera frugiperda*) cells. Recombinant baculovirus are isolated and plaque-purified. SF-9 cells (2×10^6 cells/ml) are cultured to logarithmic growth phase in TMH-FH medium (PharMingen) at 27°C, infected with recombinant baculovirus, and spent culture media
20 harvested after 3-5 days. The cells are removed from the spent culture media by centrifugation and the soluble IL-1R AcP+tag protein is detected and purified as described below.

b. Preparation of an affinity matrix composed of immobilized
25 YL1/2 antibody. Many methods can be utilized to immobilize the YL1/2 antibody to an affinity matrix including covalent crosslinking to either an activated agarose gel such as Affi-Gel 10 (BioRad Laboratories) or to an agarose gel containing immobilized Protein G (Stern and Podlaski, in: Techniques in Protein Chemistry IV, R.H.
30 Angelletti, ed., pp. 353-360, Academic Press, NY, 1993). However, for the purification of soluble IL-1R AcP, the YL1/2 antibody is covalently modified with NHS-LC-biotin (Pierce Chemical Co.) and immobilized on a streptavidin-agarose gel (Pierce Chemical Co.). YL1/2 antibody (3 mg/ml) is dialyzed against 0.1 M borate buffer, pH 8.5 followed by
35 reaction with NHS-LC-biotin at a molar ratio of 40:1 (LC-biotin:YL1/2 antibody) for 2 hrs at room temperature. The unreacted LC-biotin is quenched with 1 M glycine/0.1 M borate buffer, pH 8.4. The unreacted and quenched NHS-LC-biotin is removed by centrifugation at 1000 xg

for 15-30 min using a Centricon-30 microconcentrator (Amicon). After centrifugation, the biotinylated YL1/2 antibody is diluted with 0.1 M sodium phosphate, pH 7.0 and the process repeated two more times. Biotinylated-YL1/2 antibody (6 mg in 0.1 M sodium phosphate, pH 7.0) is reacted with streptavidin-agarose (6 ml of a 50% suspension) for 2 hrs at room temperature. The streptavidin agarose with the immobilized biotinylated YL1/2 antibody is placed into a column and washed with 10 column volumes of PBS, pH 7.4.

- 10 c. Purification of soluble IL-1R AcP. Media from either COS cells or Sf9 cells containing soluble IL-1R AcP are passed through the YL1/2 affinity column at a flow rate of 3 ml/min. The column is washed sequentially with 2 column volumes of PBS, pH 7.4, 5 column volumes of 50 mM sodium phosphate, pH 7.5, 0.5 M NaCl, 0.2 % Tween 20, 15 0.05% NaN₃ and 2 column volumes of PBS, pH 7.4. The soluble IL-1R AcP + tag is eluted with 0.1 M glycine-HCL, pH 2.8 and the fractions (1 ml) are neutralized with 3 M Tris base (0.015 ml per 1 ml fraction). The protein eluted from the column (purified soluble IL-1R AcP + tag) is characterized by reducing and non-reducing SDS-PAGE on 12% 20 acrylamide slab gels followed by silver staining to visualize the protein bands. The soluble IL-1R AcP + tag present in the conditioned media from the COS cell and baculovirus expression systems and in the purified preparations can also be identified by western blotting procedures. Proteins in the conditioned media (0.04 ml) and purified 25 soluble IL-1R AcP + tag (0.1 to 1 µg) are treated with Laemmli sample buffer without β-mercaptoethanol, separated by SDS-PAGE on 12% gels and transferred to nitrocellulose membrane (0.2 µM) as described above in Example 1. The proteins immobilized on the nitrocellulose are probed with YL1/2 antibody (5 µg/ml) and peroxidase-conjugated goat 30 anti-murine or -rat IgG antibody (1/1000 dilution) (Boehringer Mannheim Biochemicals). The immunoreactive bands are identified by ECL technique (Amersham Inc.) according to the manufacturer's protocol. The soluble IL-1R AcPs that are purified from COS cell and baculovirus expression systems should migrate as proteins of 35 approximately 65-67 kDa and 45-47 kDa, respectively.

 d. Immunization of mice and rats with soluble IL-1R AcP + tag.
Mice and rats are immunized by the i.p. and foot pad routes on days 0,

14 and 28 with 10-100 µg of soluble IL-1R AcP + tag. The protein is prepared as described in Examples 1 and 2 in Freund's complete adjuvant for the primary immunization and in Freund's incomplete adjuvant for the day 14 and 28 booster immunizations. Serum is
5 collected from the animals on day 40 and tested for antibody reactivity (see assays below). The animals are given booster immunizations (i.p., 10-25 µg of protein prepared in Freund's incomplete adjuvant) at 4 week intervals and the titer of serum antibodies determined two weeks after each immunization. When the
10 animals develop a potent serum antibody titer (e.g., 1/10⁴ dilution of the serum gives a 50% response in the EIA), they are given booster immunizations (i.v. and i.p.) of 10-100 µg of soluble IL-1R AcP + tag on two consecutive days. Three days later, spleen cells are isolated from the animal and fused with SP2/0 cells as described in Example 1 for
15 the development of the anti-murine IL-1R AcP antibodies. Hybridoma supernatants are screened for inhibitory and non-inhibitory antibodies by the assays described below. Hybridoma cell lines secreting anti-huIL-1R AcP antibodies are cloned by limiting dilution. Anti-huIL-1R AcP antibodies are purified as described in Example 1.

20

e. Assays to detect antibodies specific for human IL-1R AcP. The presence of anti-IL-1R AcP antibodies in the serum is initially determined by enzyme immunoassay (EIA) with soluble IL-1R AcP + tag immobilized on a 96 well plate. Briefly, soluble IL-1R AcP + tag (1
25 µg/ml) is diluted with 50 mM sodium carbonate buffer, pH 9.0, 0.15 M NaCl (BC saline) and passively adsorbed (100 µl, 100 ng) to the wells of a Nunc Maxisorb plate for 16 hrs at room temperature. After washing, the plates are reacted with PBS, pH 7.4, 1% bovine serum albumin (BSA) for 1 hr at 37°C. Serial dilutions [1/100 to 1/10⁶ in 50 mM
30 sodium phosphate, pH 7.5, 0.5 M NaCl, 0.1% Tween-20, 1% BSA and 0.05% NaN₃ (antibody binding buffer)] of the serum samples are incubated with the immobilized soluble IL-1R AcP for 2 hrs at room temperature. After washing the plate with PBS, pH 7.4, 0.05% Tween-20, the bound antibody is detected with peroxidase-conjugated goat
35 anti-murine or -rat IgG antibody (Boehringer-Mannheim Inc.) and visualized with TMB (tetramethylbenzidine) substrate. The color intensity in the individual wells is measured at 450 nm in a multi-

channel photometer and is proportional to the concentration of anti-IL-1R AcP antibody in the serum.

The serum antibodies are also tested for reactivity by FACS
5 (fluorescence activated cell sorting) on 1) natural huIL-1R AcP
expressed on the human cell lines YT, NC-37 and RAJI and 2)
recombinant huIL-1R AcP expressed on COS cells. Cells (1×10^6) are
incubated with serum dilutions ($1/100$ to $1/10^4$) in PBS, pH 7.4 (100
 μ l) for 1 hr at 4°C . After washing the cells with PBS, pH 7.4, to remove
10 unbound antibody, the cells are incubated with fluorescein-conjugated
goat-anti-mouse or -rat IgG antibody (Tago Laboratories) for 30 min at
 4°C . The cells are washed with PBS, pH 7.4, and the quantity of
antibody bound to the cell surface is determined by the increase in
fluorescence intensity in a FACSsort (Becton-Dickinson Co.).

15

The anti-murine IL-1R AcP antibodies 4C5 and 2E6 (Table 2)
demonstrated inhibitory and non-inhibitory activity, respectively,
against IL-1R AcP expressed on murine cells. To determine if sera
from animals immunized with human IL-1R AcP contain both
20 inhibitory and non-inhibitory antibodies, two types of assays are
performed: 1) inhibition of [^{125}I]-IL- 1β binding to human cells and 2)
immunoprecipitation of the solubilized complex of [^{125}I]-IL- 1β
crosslinked to cell surface proteins from human cells. For the inhibition
assays, serial dilutions of the sera are incubated with YT, NC-37 and
25 RAJI cells ($1-2 \times 10^6$) in binding buffer for 1 hr at room temperature.
[^{125}I]-IL- 1β (25-250 pM) is added to each tube, incubated for 3 hrs at
 4°C and cell bound radioactivity determined as previously described
in Example 1. The titer of inhibitory antibodies is determined by the
serum dilution that results in a 50% decrease in cell-bound
30 radioactivity. For the immunoprecipitation assays, dilutions of serum
are incubated for 16 hr at 4°C with the solubilized complexes of
[^{125}I]IL- 1β crosslinked to huIL-1R AcP and in the presence of
protein-G-Plus immobilized on agarose beads. Each serum sample is
tested for reactivity with solubilized complexes prepared from human
35 cell lines YT, NC-37 and RAJI. After centrifuging and washing the
protein-G-Plus agarose beads, the immunoprecipitated proteins are
analyzed by SDS-PAGE and autoradiography as described in the
Example 1 for the murine IL-1R AcP antibodies.

Immunization of mice and rats with huIL-1R AcP peptides conjugated to keyhole limpet hemocyanin (KLH)

5 Peptides corresponding to sequences 1-10, 54-64, 68-77, 265-273, 285-294, 490-499 and 505-515 of the full-length huIL-1R AcP were synthesized by standard solid phase techniques (Marglin and Merrifield, Ann. Rev. Biochem. 39: 841, 1970). The sequence of each peptide had a cysteine added to the C-terminus for the purpose of
10 covalent coupling to KLH by the MBS technique. Briefly, KLH (1.5 mg in PBS, pH 7.4) is reacted with 0.32 mg of 3-maleimidobenzoyl-N-hydroxy-succinimide ester (MBS; Boehringer Mannheim Biochemicals) for 1 hr at 4°C. The reaction mix is applied to a prepacked BioGel P10 column (10 ml) (BioRad Laboratories) and chromatographed with PBS,
15 pH 7.4. The fractions containing the KLH-MBS conjugate are pooled (2 ml) and reacted with peptide (2 mg) for 1 hr at 4°C. The KLH-peptide conjugate is concentrated in a Centricon 10 microconcentrator (Amicon) and used for immunizations. Mice and rats are immunized by the i.p. and foot pad routes on day 0, 7, 14 and 28 with 200-500 µg of
20 KLH-peptide conjugate. The conjugate is prepared in Freund's complete adjuvant for the primary immunization and Freund's incomplete adjuvant for the booster immunizations. Sera are collected from the animals on day 40 and tested for antibody reactivity in the soluble IL-1R AcP EIA. The animals are given booster immunizations (i.p., 100 µg
25 of KLH-peptide conjugate prepared in Freund's incomplete adjuvant) at 4 week intervals and the titer of serum antibodies determined two weeks after each immunization. When the animals develop a potent serum antibody titer ($1/10^4$ dilution gives a 50% response in the EIA), they are given booster immunizations with free peptide (100 µg, i.v.
30 route) and KLH-peptide conjugate (500 µg, i.p. route) on two consecutive days. Three days later, spleen cells are isolated from the animal and hybridoma cells secreting huIL-1R AcP antibodies are produced and identified as described above.

Example 16Neutralization of IL-1 β Biologic Activity by Anti-Human IL-1R AcP Antibodies and Active Fragments of IL-1R AcP

5 The ability of anti-human IL-1R AcP antibodies to neutralize IL-1 biologic activity in a dose-dependent manner can be determined in the IL-1-induced IL-6 assay with human embryonic lung fibroblast MRC-5 cells (ATCC # CCL-171). MRC-5 cells are plated in 96-well
10 cluster dishes and pretreated for 1 hr with either increasing concentrations of anti-human IL-1R AcP or active fragment of IL-1R AcP. Following the pretreatment, the cells are stimulated with either 5 pM human IL-1 α or IL-1 β for 24 hrs. The amount of IL-6 secreted by the cells in response to IL-1 is measured by a commercially available
15 IL-6 EIA (Quantikine Assay for Human IL-6, R & D Systems, Minneapolis, MN). The inhibitory effects of the antibodies and active fragments of IL-1R AcP are calculated by determining the decrease in IL-6 secretion in the presence and absence of inhibitors. For example, 5 pM and 100 pM IL-1 β stimulated the secretion of approximately
20 8100 and 9800 pg/ml of IL-6, respectively, from MRC-5 cells (Fig. 17). IL-1 receptor antagonist (IL-1RA) and anti-human Type I IL-1R antibody 4C1 blocked this IL-6 secretion in response to IL-1 β (Fig. 17). For IL-1RA and 4C1, the IC₅₀'s for blocking 5 pM IL-1 β were 200 pM and 0.025 μ g/ml, respectively (Fig. 17). The inhibition by IL-1RA
25 and 4C1 can be overridden by increasing the concentration of IL-1 β to 100 pM. With 100 pM IL-1 β , the IC₅₀'s for IL-1RA and 4C1 inhibition were >1 nM and 10 μ g/ml, respectively. These data demonstrated that the IL-1-induced IL-6 response from the MRC-5 cells was specific for IL-1 and a Type I IL-1R-dependent response, in the same
30 way that IL-1-dependent responses in murine cells are also Type I receptor-dependent (Figs. 6, 7 and 8). These IL-1 biologic assays with murine cells led to the identification of neutralizing anti-murine IL-1R AcP antibodies. Similarly, the IL-1 biologic assay with MRC-5 cells can be used to identify neutralizing anti-human IL-1R AcP antibodies
35 and active fragments of IL-1R AcP.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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15 (I) TELEX: 962292/965542 hlr ch
- (ii) TITLE OF INVENTION: HUMAN ACCESSORY PROTEIN FOR INTERLEUKIN-1
RECEPTOR
- 20 (iii) NUMBER OF SEQUENCES: 13
- (iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP .

30

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1713 base pairs
35 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 40 (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- | | | | | | | |
|------------|------------|------------|------------|------------|------------|-----|
| ATGACACTTC | TGTGGTGTGT | AGTGAGTCTC | TACTTTTATG | GAATCCTGCA | AAGTGATGCC | 60 |
| 50 | TCAGAACGCT | GCGATGACTG | GGGACTAGAC | ACCATGAGGC | AAATCCAAGT | 120 |
| | GAGCCAGCTC | GCATCAAGTG | CCCACTCTTT | GAACACTTCT | TGAAATTCAA | 180 |
| 55 | GCCCATTGAG | CTGGCCTTAC | TCTGATCTGG | TATTGGACTA | GGCAGGACCG | 240 |
| | GAGCCAATTA | ACTTCCGCCT | CCCCGAGAAC | CGCATTAGTA | AGGAGAAAGA | 300 |
| | TTCCGGCCCA | CTCTCCTCAA | TGACACTGGC | AACTATACCT | GCATGTTAAG | 360 |
| 60 | TATTGCAGCA | AAGTTGCATT | TCCCTTGGAA | GTTGTTCAAA | AAGACAGCTG | 420 |
| | TTTCAATTCC | | | | | |

CCCATGAAAC TCCCAGTGCA TAAACTGTAT ATAGAATATG GCATTCAGAG GATCACTTGT 480
CCAAATGTAG ATGGATATTT TCCTTCCAGT GTCAAACCGA CTATCACTTG GTATATGGGC 540
5 TGTTATAAAA TACAGAATTT TAATAATGTA ATACCCGAAG GTATGAACTT GAGTTTCCTC 600
ATTGCCTTAA TTTCAAATAA TGGAAATTAC ACATGTGTTG TTACATATCC AGAAAATGGA 660
10 CGTACGTTTC ATCTCACCAG GACTCTGACT GTAAAGGTAG TAGGCTCTCC AAAAAATGCA 720
GTGCCCCCTG TGATCCATTC ACCTAATGAT CATGTGGTCT ATGAGAAAGA ACCAGGAGAG 780
GAGCTACTCA TTCCCTGTAC GGTCTATTTT AGTTTTCTGA TGGATTCTCG CAATGAGGTT 840
15 TGGTGGACCA TTGATGGAAA AAAACCTGAT GACATCACTA TTGATGTCAC CATTAACGAA 900
AGTATAAGTC ATAGTAGAAC AGAAGATGAA ACAAGAACTC AGATTTTGAG CATCAAGAAA 960
20 GTTACCTCTG AGGATCTCAA GCGCAGCTAT GTCTGTCATG CTAGAAGTGC CAAAGGCGAA 1020
GTTGCCAAAG CAGCCAAGGT GACGCAGAAA GTGCCAGCTC CAAGATACAC AGTGGAAGTG 1080
GCTTGTGGTT TTGGAGCCAC AGTCCTGCTA GTGGTGATTC TCATTGTTGT TTACCATGTT 1140
25 TACTGGCTAG AGATGGTCCT ATTTTACCGG GCTCATTTTG GAACAGATGA AACCATTTTA 1200
GATGGAAAAG AGTATGATAT TTATGTATCC TATGCAAGGA ATGCGGAAGA AGAAGAATTT 1260
30 GTTTTACTGA CCTCCGTGG AGTTTTGGAG AATGAATTTG GATACAAGCT GTGCATCTTT 1320
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35 CTGGAGCTCA AGGCTGGCCT AGAAAATATG GGCTCTCGGG GCAACATCAA CGTCATTTTA 1500
GTACAGTACA AAGCTGTGAA GGAAACGAAG GTGAAAGAGC TGAAGAGGGC TAAGACGGTG 1560
CTCACGGTCA TTAAATGGAA AGGGGAAAAA TCCAAGTATC CACAGGGCAG GTTCTGGAAG 1620
40 CAGCTGCAGG TGGCCATGCC AGTGAAGAAA AGTCCCAGGC GGTCTAGCAG TGATGAGCAG 1680
GGCCTCTCGT ATTCATCTTT GAAAAATGTA TGA 1713

45 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1713 base pairs
(B) TYPE: nucleic acid
50 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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5	CTCGGTCGAG	CGTAGTTCAC	GGGTGAGAAA	CTTGTGAAGA	ACTTTAAGTT	GATGTCGTGT	180
	CGGGTAAGTC	GACCGGAATG	AGACTAGACC	ATAACCTGAT	CCGTCCTGGC	CCTGGAACTC	240
10	CTCGGTAAAT	TGAAGGCGGA	GGGGCTCTTG	GCGTAATCAT	TCCTCTTTCT	ACACGACACC	300
	AAGGCCGGGT	GAGAGGAGTT	ACTGTGACCG	TTGATATGGA	CGTACAATTC	CTTGTGATGT	360
	ATAACGTCGT	TTCAACGTAA	AGGGAACCTT	CAACAAGTTT	TTCTGTCGAC	AAAGTTAAGG	420
15	GGGTACTTTG	AGGGTCACGT	ATTTGACATA	TATCTTATAC	CGTAAGTCTC	CTAGTGAACA	480
	GGTTTACATC	TACCTATAAA	AGGAAGGTCA	CAGTTTGGCT	GATAGTGAAC	CATATACCCG	540
20	ACAATATTTT	ATGTCTTAAA	ATTATTACAT	TATGGGCTTC	CATACTTGAA	CTCAAAGGAG	600
	TAACGGAATT	AAAGTTTATT	ACCTTTAATG	TGTACACAAC	AATGTATAGG	TCTTTTACCT	660
	GCATGCAAAG	TAGAGTGGTC	CTGAGACTGA	CATTTCCATC	ATCCGAGAGG	TTTTTTACGT	720
25	CACGGGGGAC	ACTAGGTAAG	TGGATTACTA	GTACACCAGA	TACTCTTTCT	TGGTCTCTC	780
	CTCGATGAGT	AAGGGACATG	CCAGATAAAA	TCAAAAGACT	ACCTAAGAGC	GTTACTCCAA	840
30	ACCACCTGGT	AACTACCTTT	TTTTGGACTA	CTGTAGTGAT	AACTACAGTG	GTAATTGCTT	900
	TCATATTCAG	TATCATCTTG	TCTTCTACTT	TGTTCTTGAG	TCTAAAACTC	GTAGTTCTTT	960
	CAATGGAGAC	TCCTAGAGTT	CGCGTCGATA	CAGACAGTAC	GATCTTCACG	GTTTCCGCTT	1020
35	CAACGGTTTC	GTCGGTTCCA	CTGCGTCTTT	CACGGTCGAG	GTTCTATGTG	TCACCTTGAC	1080
	CGAACACCAA	AACCTCGGTG	TCAGGACGAT	CACCACTAAG	AGTAACAACA	AATGGTACAA	1140
40	ATGACCGATC	TCTACCAGGA	TAAAATGGCC	CGAGTAAAC	CTTGTCTACT	TTGGTAAAAT	1200
	CTACCTTTTC	TCATACTATA	AATACATAGG	ATACGTTCTT	TACGCCTTCT	TCTTCTTAAA	1260
	CAAAATGACT	GGGAGGCACC	TCAAAACCTC	TTACTTAAAC	CTATGTTTGA	CACGTAGAAA	1320
45	CTGGCTCTGT	CAGACGGACC	CCCTTAACAG	TGTCTACTCT	GAAACTCGAA	GTAAGTCTTT	1380
	TCGTCTGCGG	AGGACCAACA	AGATTGCGGG	TTGATGCACG	AGGTCCCTTG	GGTTCGGGAG	1440
50	GACCTCGAGT	TCCGACCGGA	TCTTTTATAC	CCGAGAGCCC	CGTTGTAGTT	GCAGTAAAAT	1500
	CATGTCATGT	TTGACACTT	CCTTTGCTTC	CACTTTCTCG	ACTTCTCCCG	ATTCTGCCAC	1560
	GAGTGCCAGT	AATTTACCTT	TCCCCTTTTT	AGGTTTCATAG	GTGTCCCGTC	CAAGACCTTC	1620
55	GTCGACGTCC	ACCGGTACGG	TCACTTCTTT	TCAGGGTCCG	CCAGATCGTC	ACTACTCGTC	1680
	CCGGAGAGCA	TAAGTAGAAA	CTTTTTACAT	ACT			1713

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 570 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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 1 5 10 15
 Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met
 20 25 30
 25 Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro
 35 40 45
 Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala
 50 55 60
 30 Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu
 65 70 75 80
 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys
 85 90 95
 35 Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr
 100 105 110
 40 Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro
 115 120 125
 Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu
 130 135 140
 45 Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys
 145 150 155 160
 Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr
 165 170 175
 50 Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro
 180 185 190
 55 Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly
 195 200 205
 Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His
 210 215 220
 60 Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala
 225 230 235 240

- 75 -

	Val	Pro	Pro	Val	Ile	His	Ser	Pro	Asn	Asp	His	Val	Val	Tyr	Glu	Lys	
					245					250					255		
5	Glu	Pro	Gly	Glu	Glu	Leu	Leu	Ile	Pro	Cys	Thr	Val	Tyr	Phe	Ser	Phe	
				260					265					270			
	Leu	Met	Asp	Ser	Arg	Asn	Glu	Val	Trp	Trp	Thr	Ile	Asp	Gly	Lys	Lys	
			275					280					285				
10	Pro	Asp	Asp	Ile	Thr	Ile	Asp	Val	Thr	Ile	Asn	Glu	Ser	Ile	Ser	His	
		290					295					300					
	Ser	Arg	Thr	Glu	Asp	Glu	Thr	Arg	Thr	Gln	Ile	Leu	Ser	Ile	Lys	Lys	
	305					310					315					320	
15	Val	Thr	Ser	Glu	Asp	Leu	Lys	Arg	Ser	Tyr	Val	Cys	His	Ala	Arg	Ser	
				325						330					335		
	Ala	Lys	Gly	Glu	Val	Ala	Lys	Ala	Ala	Lys	Val	Thr	Gln	Lys	Val	Pro	
20				340					345					350			
	Ala	Pro	Arg	Tyr	Thr	Val	Glu	Leu	Ala	Cys	Gly	Phe	Gly	Ala	Thr	Val	
		355					360						365				
25	Leu	Leu	Val	Val	Ile	Leu	Ile	Val	Val	Tyr	His	Val	Tyr	Trp	Leu	Glu	
	370					375						380					
	Met	Val	Leu	Phe	Tyr	Arg	Ala	His	Phe	Gly	Thr	Asp	Glu	Thr	Ile	Leu	
	385					390				395						400	
30	Asp	Gly	Lys	Glu	Tyr	Asp	Ile	Tyr	Val	Ser	Tyr	Ala	Arg	Asn	Ala	Glu	
				405						410					415		
	Glu	Glu	Glu	Phe	Val	Leu	Leu	Thr	Leu	Arg	Gly	Val	Leu	Glu	Asn	Glu	
35				420					425					430			
	Phe	Gly	Tyr	Lys	Leu	Cys	Ile	Phe	Asp	Arg	Asp	Ser	Leu	Pro	Gly	Gly	
		435						440				445					
40	Ile	Val	Thr	Asp	Glu	Thr	Leu	Ser	Phe	Ile	Gln	Lys	Ser	Arg	Arg	Leu	
	450						455					460					
	Leu	Val	Val	Leu	Ser	Pro	Asn	Tyr	Val	Leu	Gln	Gly	Thr	Gln	Ala	Leu	
	465					470					475					480	
45	Leu	Glu	Leu	Lys	Ala	Gly	Leu	Glu	Asn	Met	Gly	Ser	Arg	Gly	Asn	Ile	
				485					490						495		
	Asn	Val	Ile	Leu	Val	Gln	Tyr	Lys	Ala	Val	Lys	Glu	Thr	Lys	Val	Lys	
50				500					505					510			
	Glu	Leu	Lys	Arg	Ala	Lys	Thr	Val	Leu	Thr	Val	Ile	Lys	Trp	Lys	Gly	
		515						520					525				
55	Glu	Lys	Ser	Lys	Tyr	Pro	Gln	Gly	Arg	Phe	Trp	Lys	Gln	Leu	Gln	Val	
	530						535					540					
	Ala	Met	Pro	Val	Lys	Lys	Ser	Pro	Arg	Arg	Ser	Ser	Ser	Asp	Glu	Gln	
	545					550					555					560	
60	Gly	Leu	Ser	Tyr	Ser	Ser	Leu	Lys	Asn	Val							
				565					570								

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1713 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

20 ATGGGACTTC TGTGGTATTT GATGAGTCTG TCCTTCTATG GGATCCTGCA GAGTCATGCT 60
TCGGAGCGCT GTGATGACTG GGGACTAGAT ACCATGCGAC AAATCCAAGT GTTTGAAGAT 120
GAGCCGGCTC GAATCAAGTG CCCCCTCTTT GAACACTTCC TGAAGTACAA CTACAGCACT 180
25 GCCCATTCCCT CTGGCCTTAC CCTGATCTGG TACTGGACCA GGCAAGACCG GGACCTGGAG 240
GAGCCCATTA ACTTCCGCCT CCCAGAGAAT CGCATCAGTA AGGAGAAAGA TGTGCTCTGG 300
TTCCGGCCCA CCTCCTCAA TGACACGGGC AATTACACCT GCATGTTGAG GAACACAAC 360
30 TACTGCAGCA AAGTTGCATT TCCCCTGGAA GTTGTTCAGA AGGACAGCTG TTTCAATTCT 420
GCCATGAGAT TCCAGTGCA CAAGATGTAT ATTGAACATG GCATTCATAA GATCACATGT 480
35 CCAAATGTAG ACGGATACTT TCCTTCCAGT GTCAAACCAT CGGTCACTTG GTATAAGGGT 540
TGTA CTGAAA TAGTGGACTT TCATAATGTA CTACCCGAGG GCATGAACTT GAGCTTTTTTC 600
ATCCCCCTGG TTTCAAATAA CGGAAATTAC ACATGTGTGG TTACATATCC TGAAAACGGA 660
40 CGTCTCTTTC ACCTCACCAG GACTGTGACT GTAAAGGTGG TGGGCTCACC AAAGGATGCA 720
TTGCCACCCC AGATCTATTC TCCAAATGAC CGTGTGTCT ATGAGAAAGA ACCAGGAGAG 780
45 GAACTGGTTA TTCCCTGCAA AGTCTATTTC AGTTTCATTA TGGACTCCCA CAATGAGGTC 840
TGGTGGACCA TTGATGGAAG GAAGCCTGAT GACGTCACAG TCGACATCAC TATTAATGAA 900
AGTGTAAGTT ATTCTTCAAC GGAAGATGAA ACAAGGACTC AGATTTTGAG CATCAAGAAA 960
50 GTCACCCCGG AGGATCTCAG GCGCAACTAT GTCTGTCATG CTCGAAATAC CAAAGGGGAA 1020
GCTGAGCAGG CTGCCAAGGT GAAACAGAAA GTCATACCAC CAAGGTACAC AGTAGAAGTC 1080
55 GCCTGTGGTT TTGGAGCCAC GGTCTTTCTG GTAGTGGTTC TCATTGTGGT TTACCATGTT 1140
TACTGGCTGG AGATGGTCCT CTTTTACCGA GCTCACTTTG GAACAGATGA AACAAATCTT 1200
GATGGAAAGG AGTATGATAT TTATGTTTCC TATGCAAGAA ATGTGGAAGA AGAGGAATTT 1260
60 GTGCTGCTGA CGCTGCGTGG AGTTTGGAG AATGAGTTTG GATACAAGCT GTGCATCTTC 1320
GACAGAGACA GCCTGCCTGG GGAATTGTC ACAGATGAGA CCCTGAGCTT CATTCAAGAA 1380

AGCAGACGAC TCCTGGTTGT CCTAAGTCCC AACTACGTGC TCCAGGGAAC ACAAGCCCTC 1440
 CTGGAGCTCA AGGCTGGCCT AGAAAATATG GCCTCCCGGG GCAACATCAA CGTCATTTTA 1500
 5 GTGCAGTACA AAGCTGTGAA GGACATGAAG GTGAAAGAGC TGAAGCGGGC TAAGACGGTG 1560
 CTCACGGTCA TTAAATGGAA AGGAGAGAAA TCCAAGTATC CTCAGGGCAG GTTCTGGAAG 1620
 10 CAGTTGCAGG TGGCCATGCC AGTGAAGAAG AGTCCCAGGT GGTCTAGCAA TGACAAGCAG 1680
 GGTCTCTCCT ACTCATCCCT GAAAAACGTA TGA 1713

(2) INFORMATION FOR SEQ ID NO:5:

15

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1713 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25

(iv) ANTI-SENSE: YES

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TACCCTGAAG ACACCATAAA CTACTCAGAC AGGAAGATAC CCTAGGACGT CTCAGTACGA 60
 AGCCTCGCGA CACTACTGAC CCCTGATCTA TGGTACGCTG TTTAGGTTCA CAAACTTCTA 120
 35 CTCGGCCGAG CTTAGTTCAC GGGGGAGAAA CTTGTGAAGG ACTTCATGTT GATGTCGTGA 180
 CGGGTAAGGA GACCGGAATG GGACTAGACC ATGACCTGGT CCGTTCTGGC CCTGGACCTC 240
 40 CTCGGGTAAT TGAAGGCGGA GGGTCTCTTA GCGTAGTCAT TCCTCTTTCT ACACGAGACC 300
 AAGGCCGGGT GGGAGGAGTT ACTGTGCCCC TTAATGTGGA CGTACAATC CTTGTGTTGA 360
 ATGACGTCGT TTCAACGTAA AGGGGACCTT CAACAAGTCT TCCTGTCGAC AAAGTTAAGA 420
 45 CGGTACTCTA AGGGTCACGT GTTCTACATA TAACTGTAC CGTAAGTATT CTAGTGTA 480
 GGTTTACATC TGCCTATGAA AGGAAGGTCA CAGTTTGGTA GCCAGTGAAC CATATTCCCA 540
 50 ACATGACTTT ATCACCTGAA AGTATTACAT GATGGGCTCC CGTACTTGAA CTCGAAAAAG 600
 TAGGGGAACC AAAGTTTATT GCCTTTAATG TGTACACACC AATGTATAGG ACTTTTGCCT 660
 GCAGAGAAAG TGGAGTGGTC CTGACACTGA CATTTCCACC ACCCGAGTGG TTTCCTACGT 720
 55 AACGGTGGGG TCTAGATAAG AGGTTTACTG GCACAACAGA TACTCTTTCT TGGTCCTCTC 780
 CTTGACCAAT AAGGGACGTT TCAGATAAAG TCAAAGTAAT ACCTGAGGGT GTTACTCCAG 840
 60 ACCACCTGGT AACTACCTTT CTTGCGACTA CTGCAGTGTC AGCTGTAGTG ATAATTACTT 900
 TCACATTCAA TAAGAAGTTG CCTTCTACTT TGTCCTGAG TCTAAACTC GTAGTTCTTT 960

CAGTGGGGCC TCCTAGAGTC CGCGTTGATA CAGACAGTAC GAGCTTTATG GTTCCCTTT 1020
 CGACTCGTCC GACGGTTCCA CTTTGTCTTT CAGTATGGTG GTTCCATGTG TCATCTTGAG 1080
 5 CGGACACCAA AACCTCGGTG CCAGAAAGAC CATCACCAAG AGTAACACCA AATGGTACAA 1140
 ATGACCGACC TCTACCAGGA GAAAATGGCT CGAGTGAAAC CTTGTCTACT TTGTTAAGAA 1200
 CTACCTTTCC TCATACTATA AATACAAAGG ATACGTTCTT TACACCTTCT TCTCCTTAAA 1260
 10 CACGACGACT GCGACGCACC TCAAAACCTC TTA CTCAAAC CTATGTTTGA CACGTAGAAG 1320
 CTGTCTCTGT CGGACGGACC CCCTTAACAG TGTCTACTCT GGGACTCGAA GTAAGTCTTT 1380
 15 TCGTCTGCTG AGGACCAACA GGATTCAGGG TTGATGCACG AGGTCCCTTG TGTTCTGGGAG 1440
 GACCTCGAGT TCCGACCGGA TCTTTTATAC CGGAGGGCCC CGTTGTAGTT GCAGTAAAAT 1500
 CACGTCATGT TTCGACACTT CCTGTACTTC CACTTTCTCG ACTTCGCCCCG ATTCTGCCAC 1560
 20 GAGTGCCAGT AATTTACCTT TCCTCTCTTT AGGTTCATAG GACTCCCGTC CAAGACCTTC 1620
 GTCAACGTCC ACCGGTACGG TCACTTCTTC TCAGGGTCCA CCAGATCGTT ACTGTTCTGC 1680
 25 CCAGAGAGGA TGAGTAGGGA CTTTTTGCAT ACT 1713

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 30 (A) LENGTH: 570 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

45 Met Gly Leu Leu Trp Tyr Leu Met Ser Leu Ser Phe Tyr Gly Ile Leu
 1 5 10 15
 Gln Ser His Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met
 20 25 30
 50 Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro
 35 40 45
 55 Leu Phe Glu His Phe Leu Lys Tyr Asn Tyr Ser Thr Ala His Ser Ser
 50 55 60
 Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu
 65 70 75 80
 60 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys
 85 90 95

	Asp	Val	Leu	Trp	Phe	Arg	Pro	Thr	Leu	Leu	Asn	Asp	Thr	Gly	Asn	Tyr	
			100						105					110			
5	Thr	Cys	Met	Leu	Arg	Asn	Thr	Thr	Tyr	Cys	Ser	Lys	Val	Ala	Phe	Pro	
			115					120					125				
	Leu	Glu	Val	Val	Gln	Lys	Asp	Ser	Cys	Phe	Asn	Ser	Ala	Met	Arg	Phe	
		130					135					140					
10	Pro	Val	His	Lys	Met	Tyr	Ile	Glu	His	Gly	Ile	His	Lys	Ile	Thr	Cys	
	145					150					155					160	
	Pro	Asn	Val	Asp	Gly	Tyr	Phe	Pro	Ser	Ser	Val	Lys	Pro	Ser	Val	Thr	
					165					170					175		
15	Trp	Tyr	Lys	Gly	Cys	Thr	Glu	Ile	Val	Asp	Phe	His	Asn	Val	Leu	Pro	
				180					185					190			
	Glu	Gly	Met	Asn	Leu	Ser	Phe	Phe	Ile	Pro	Leu	Val	Ser	Asn	Asn	Gly	
20			195					200					205				
	Asn	Tyr	Thr	Cys	Val	Val	Thr	Tyr	Pro	Glu	Asn	Gly	Arg	Leu	Phe	His	
		210					215					220					
25	Leu	Thr	Arg	Thr	Val	Thr	Val	Lys	Val	Val	Gly	Ser	Pro	Lys	Asp	Ala	
	225					230					235					240	
	Leu	Pro	Pro	Gln	Ile	Tyr	Ser	Pro	Asn	Asp	Arg	Val	Val	Tyr	Glu	Lys	
				245					250						255		
30	Glu	Pro	Gly	Glu	Glu	Leu	Val	Ile	Pro	Cys	Lys	Val	Tyr	Phe	Ser	Phe	
			260					265						270			
	Ile	Met	Asp	Ser	His	Asn	Glu	Val	Trp	Trp	Thr	Ile	Asp	Gly	Lys	Lys	
35			275				280						285				
	Pro	Asp	Asp	Val	Thr	Val	Asp	Ile	Thr	Ile	Asn	Glu	Ser	Val	Ser	Tyr	
		290					295					300					
40	Ser	Ser	Thr	Glu	Asp	Glu	Thr	Arg	Thr	Gln	Ile	Leu	Ser	Ile	Lys	Lys	
	305					310					315					320	
	Val	Thr	Pro	Glu	Asp	Leu	Arg	Arg	Asn	Tyr	Val	Cys	His	Ala	Arg	Asn	
				325						330					335		
45	Thr	Lys	Gly	Glu	Ala	Glu	Gln	Ala	Ala	Lys	Val	Lys	Gln	Lys	Val	Ile	
			340					345						350			
	Pro	Pro	Arg	Tyr	Thr	Val	Glu	Leu	Ala	Cys	Gly	Phe	Gly	Ala	Thr	Val	
50			355				360						365				
	Phe	Leu	Val	Val	Val	Leu	Ile	Val	Val	Tyr	His	Val	Tyr	Trp	Leu	Glu	
		370				375						380					
55	Met	Val	Leu	Phe	Tyr	Arg	Ala	His	Phe	Gly	Thr	Asp	Glu	Thr	Ile	Leu	
	385					390					395					400	
	Asp	Gly	Lys	Glu	Tyr	Asp	Ile	Tyr	Val	Ser	Tyr	Ala	Arg	Asn	Val	Glu	
				405						410					415		
60	Glu	Glu	Glu	Phe	Val	Leu	Leu	Thr	Leu	Arg	Gly	Val	Leu	Glu	Asn	Glu	
			420					425						430			

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Phe Gly Tyr Lys Leu Cys Ile Phe Asp Arg Asp Ser Leu Pro Gly Gly
 435 440 445
 5 Ile Val Thr Asp Glu Thr Leu Ser Phe Ile Gln Lys Ser Arg Arg Leu
 450 455 460
 Leu Val Val Leu Ser Pro Asn Tyr Val Leu Gln Gly Thr Gln Ala Leu
 465 470 475 480
 10 Leu Glu Leu Lys Ala Gly Leu Glu Asn Met Ala Ser Arg Gly Asn Ile
 485 490 495
 Asn Val Ile Leu Val Gln Tyr Lys Ala Val Lys Asp Met Lys Val Lys
 500 505 510
 15 Glu Leu Lys Arg Ala Lys Thr Val Leu Thr Val Ile Lys Trp Lys Gly
 515 520 525
 Glu Lys Ser Lys Tyr Pro Gln Gly Arg Phe Trp Lys Gln Leu Gln Val
 530 535 540
 20 Ala Met Pro Val Lys Lys Ser Pro Arg Trp Ser Ser Asn Asp Lys Gln
 545 550 555 560
 25 Gly Leu Ser Tyr Ser Ser Leu Lys Asn Val
 565 570

(2) INFORMATION FOR SEQ ID NO:7:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1077 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

45 ATGACACTTC TGTGGTGTGT AGTGAGTCTC TACTTTTATG GAATCCTGCA AAGTGATGCC 60
 TCAGAACGCT GCGATGACTG GGGACTAGAC ACCATGAGGC AAATCCAAGT GTTTGAAGAT 120
 50 GAGCCAGCTC GCATCAAGTG CCCACTCTTT GAACACTTCT TGAAATTCAA CTACAGCACA 180
 GCCCATTCAG CTGGCCTTAC TCTGATCTGG TATTGGACTA GGCAGGACCG GGACCTTGAG 240
 GAGCCAATTA ACTTCCGCCT CCCCAGAGAAC CGCATTAGTA AGGAGAAAGA TGTGCTGTGG 300
 55 TTCCGGCCCA CTCTCCTCAA TGACACTGGC AACTATACCT GCATGTTAAG GAACACTACA 360
 TATTGCAGCA AAGTTGCATT TCCCTTGGA GTTGTTCAA AAGACAGCTG TTTCAATTCC 420
 60 CCCATGAAAC TCCAGTGCA TAACTGTAT ATAGAATATG GCATTGAGAG GATCACTTGT 480
 CCAAATGTAG ATGGATATTT TCCTTCCAGT GTCAAACCGA CTATCACTTG GTATATGGGC 540

TGTTATAAAA TACAGAATTT TAATAATGTA ATACCCGAAG GTATGAACTT GAGTTTCCTC 600
ATTGCCTTAA TTTCAAATAA TGGAAATTAC ACATGTGTTG TTACATATCC AGAAAATGGA 660
5 CGTACGTTTC ATCTCACCAG GACTCTGACT GTAAAGGTAG TAGGCTCTCC AAAAAATGCA 720
GTGCCCCCTG TGATCCATTC ACCTAATGAT CATGTGGTCT ATGAGAAAGA ACCAGGAGAG 780
GAGCTACTCA TTCCCTGTAC GGTCTATTTT AGTTTTCTGA TGGATTCTCG CAATGAGGTT 840
10 TGGTGGACCA TTGATGGAAA AAAACCTGAT GACATCACTA TTGATGTCAC CATTAACGAA 900
AGTATAAGTC ATAGTAGAAC AGAAGATGAA ACAAGAACTC AGATTTTGAG CATCAAGAAA 960
15 GTTACCTCTG AGGATCTCAA GCGCAGCTAT GTCTGTCATG CTAGAAGTGC CAAAGGCGAA 1020
GTTGCCAAAG CAGCCAAGGT GACGCAGAAA GTGCCAGCTC CAAGATACAC AGTGGA 1077

(2) INFORMATION FOR SEQ ID NO:8:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1077 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30

(iv) ANTI-SENSE: YES

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TACTGTGAAG ACACCACACA TCACTCAGAG ATGAAAATAC CTTAGGACGT TTCACTACGG 60
AGTCTTGCGA CGCTACTGAC CCCTGATCTG TGGTACTCCG TTTAGGTTCA CAAACTTCTA 120
40 CTCGGTCGAG CGTAGTTCAC GGGTGAGAAA CTTGTGAAGA ACTTTAAGTT GATGTCGTGT 180
CGGGTAAGTC GACCGGAATG AGACTAGACC ATAACCTGAT CCGTCCTGGC CCTGGAAGTC 240
45 CTCGGTTAAT TGAAGGCGGA GGGGCTCTTG GCGTAATCAT TCCTCTTTCT ACACGACACC 300
AAGGCCGGGT GAGAGGAGTT ACTGTGACCG TTGATATGGA CGTACAATTC CTTGTGATGT 360
ATAACGTCGT TTCAACGTAA AGGGAACCTT CAACAAGTTT TTCTGTCGAC AAAGTTAAGG 420
50 GGGTACTTTG AGGGTCACGT ATTTGACATA TATCTTATAC CGTAAGTCTC CTAGTGAACA 480
GGTTTACATC TACCTATAAA AGGAAGGTCA CAGTTTGGCT GATAGTGAAC CATATACCCG 540
55 ACAATATTTT ATGTCTTAAA ATTATTACAT TATGGGCTTC CATACTTGAA CTCAAAGGAG 600
TAACGGAATT AAAGTTTATT ACCTTTAATG TGTACACAAC AATGTATAGG TCTTTTACCT 660
GCATGCAAAG TAGAGTGGTC CTGAGACTGA CATTTCCATC ATCCGAGAGG TTTTTTACGT 720
60 CACGGGGGAC ACTAGGTAAG TGGATTACTA GTACACCAGA TACTCTTTCT TGGTCTCTC 780
CTCGATGAGT AAGGGACATG CCAGATAAAA TCAAAAGACT ACCTAAGAGC GTTACTCCAA 840

ACCACCTGGT AACTACCTTT TTTTGGACTA CTGTAGTGAT AACTACAGTG GTAATTGCTT 900
 TCATATTCAG TATCATCTTG TCTTCTACTT TGTTCCTGAG TCTAAACTC GTAGTTCTTT 960
 5 CAATGGAGAC TCCTAGAGTT CGCGTCGATA CAGACAGTAC GATCTTCACG GTTCCGCTT 1020
 CAACGGTTTC GTCGGTTCCA CTGCGTCTTT CACGGTCGAG GTTCTATGTG TCACCTT 1077

10 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 359 amino acids
 (B) TYPE: amino acid
 15 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Thr Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu
 1 5 10 15
 30 Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met
 20 25 30
 Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro
 35 35 40 45
 Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala
 50 55 60
 40 Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu
 65 70 75 80
 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys
 85 90 95
 45 Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr
 100 105 110
 Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro
 50 115 120 125
 Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu
 130 135 140
 55 Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys
 145 150 155 160
 Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr
 165 170 175
 60 Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro
 180 185 190

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Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly
 195 200 205
 5 Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His
 210 215 220
 Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala
 225 230 235 240
 10 Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys
 245 250 255
 Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe
 260 265 270
 15 Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys
 275 280 285
 Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His
 290 295 300
 Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys
 305 310 315 320
 25 Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser
 325 330 335
 Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Thr Gln Lys Val Pro
 340 345 350
 30 Ala Pro Arg Tyr Thr Val Glu
 355

(2) INFORMATION FOR SEQ ID NO:10:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGCCGGATCC ATGACACTTC TGTGGTGTGT AGTGAGTCTC TAC

43

(2) INFORMATION FOR SEQ ID NO:11:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 61 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

10 CGCGCGGGTA CCCTAGAACT CTCAGCTTC CACTGTGTAT CTTGGAGCTG GCACTTTCTG 60
c 61

(2) INFORMATION FOR SEQ ID NO:12:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

30 GATCCAGAAT TCATAATAG 19

(2) INFORMATION FOR SEQ ID NO:13:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

50 GTCTTAAGTA TTATCCATG 19

Claims

1. A polynucleotide which encodes an IL-1 receptor accessory protein or an active fragment thereof.
5
2. A polynucleotide of claim 1 comprising a DNA sequence selected from
 - (a) a polynucleotide having essentially the sequence [SEQ ID NO:1]; or
 - 10 (b) a polynucleotide which hybridizes to the DNA of (a) under moderately stringent conditions; or
 - (c) a polynucleotide which differs in codon sequence due to the degeneracy of the genetic code.
- 15 3. A polynucleotide of claim 1 or claim 2 which encodes a human IL-1 receptor accessory protein.
4. A polynucleotide of claim 3 which encodes the human IL-1 receptor protein having the amino acid sequence [SEQ ID NO:3] or an
20 active fragment thereof.
5. A polynucleotide of claim 4 having the sequence [SEQ ID NO:1]
- 25 6. A polynucleotide of claim 1 or claim 2 which encodes a soluble IL-1 receptor accessory protein.
7. A polynucleotide of claim 6 which encodes a human soluble IL-1 receptor accessory protein.
30
8. A polynucleotide of claim 7 which encodes the human soluble IL-1 receptor protein having the amino acid sequence [SEQ ID NO:9] or an active fragment thereof.
- 35 9. A polynucleotide of claim 8 having the sequence [SEQ ID NO:7].

10. A polynucleotide of claim 1 or claim 2 which is an antisense polynucleotide.
- 5 11. A vector which comprises a polynucleotide according to any of claims 1 to 10.
12. A vector of claim 11 which is an expression vector.
- 10 13. A host cell which comprises a vector of claim 11 or claim 12.
14. The IL-1 receptor accessory protein or an active fragment thereof.
- 15 15. A protein of claim 14 encoded by a polynucleotide as defined in claim 2.
16. A protein according to claim 14 or claim 15 which is the
- 20 human IL-1 receptor accessory protein.
17. A protein of claim 16 which has the amino acid sequence [SEQ ID NO:3].
- 25 18. A protein according to claim 14 or claim 15 which is a soluble human IL-1 receptor accessory protein.
19. A protein of claim 18 having the amino acid sequence [SEQ ID NO:9].
- 30 20. A protein according to any of claims 14 to 19 carrying one or more side groups which have been modified.
21. An antibody which binds specifically to the human IL-1
- 35 receptor accessory protein and prevents activation of the IL-1 receptor complex by IL-1.
22. An antibody of claim 21 which is a monoclonal antibody.

23. An antibody according to claim 21 or claim 22 having a binding affinity to the IL-1 receptor accessory complex of from about K_D 0.1 nM to about K_D 10 nM.

5 24. A pharmaceutical composition which comprises a compound according to any of claims 10 and 14 to 23 and a pharmaceutically acceptable carrier.

10 25. A pharmaceutical composition according to claim 24 in combination with one or more other cytokine antagonists.

26. A process for the preparation of an IL-1 receptor accessory protein comprising the steps of:

- 15 (a) expressing a polypeptide encoded by a DNA according to any of claims 1 to 10 in a suitable host,
 (b) isolating said IL-1 receptor accessory protein, and
 (c) if desired, converting it in an analogue wherein one or more side groups are modified.

20 27. A process for the preparation of an IL-1 receptor accessory protein antibody comprising the steps of:

- (a) preparation of a hybridoma cell line producing a monoclonal antibody which specifically binds to the IL-1 receptor accessory protein and
25 (b) production and isolation of the monoclonal antibody.

28. A compound as claimed in any one of claims 14 to 23 prepared by a process as claimed in claim 26 or claim 27.

30 29. A compound according to any of claims 10 and 14 to 23 for use as therapeutically active substance.

35 30. A compound according to any of claims 10 and 14 to 23 for use in the treatment of inflammatory or immune responses and/or for regulating and preventing inflammatory or immunological activities of Interleukin-1.

31. A compound according to any of claims 10 and 14 to 23 in the treatment of acute or chronic diseases, preferably rheumatoid arthritis, inflammatory bowel disease, septic shock, transplant
5 rejection, psoriasis, asthma and Type I diabetes or in the treatment of cancer, preferably acute and chronic myelogenous leukemia.

32. The use of a compound according to any of claims 10 and 14 to 23 for the manufacture of a medicament for the control or
10 prevention of illness.

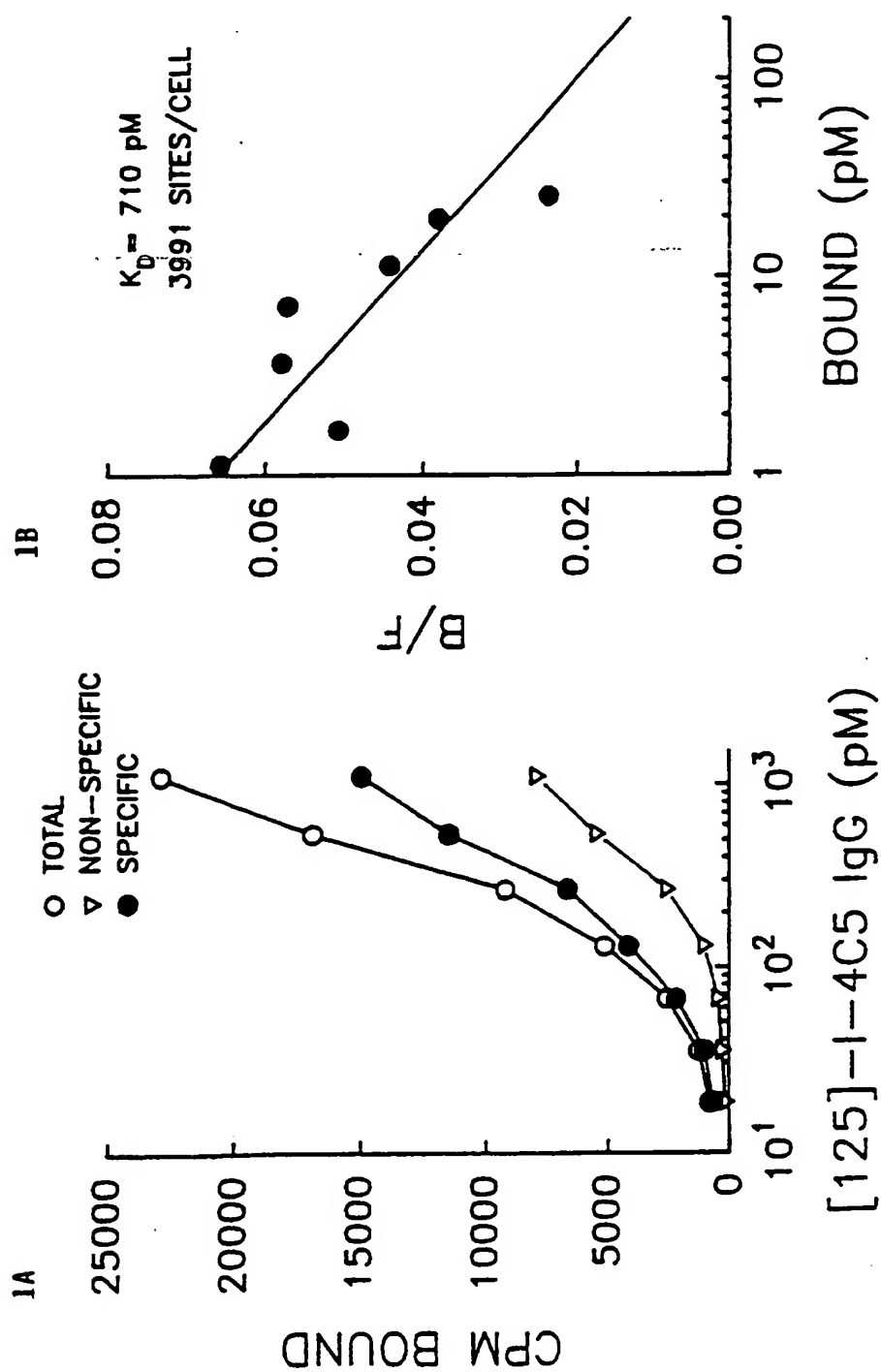
33. The use of a compound of claim 10 and 14 to 23 for the manufacture of a medicament for the treatment of inflammatory or immune responses and/or for regulating and preventing
15 inflammatory or immunological activities of Interleukin-1.

34. The use of a compound of claims 10 and 14 to 23 for the manufacture of a medicament for the treatment or prophylaxis of rheumatoid arthritis, inflammatory bowel disease, septic shock,
20 transplant rejection, psoriasis, asthma and Type I diabetes or for the treatment or prophylaxis of cancer, preferably acute and chronic myelogenous leukemia.

35. The novel compounds, compositions, processes and uses
25 thereof substantially as described herein.

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Fig. 1



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Fig. 2

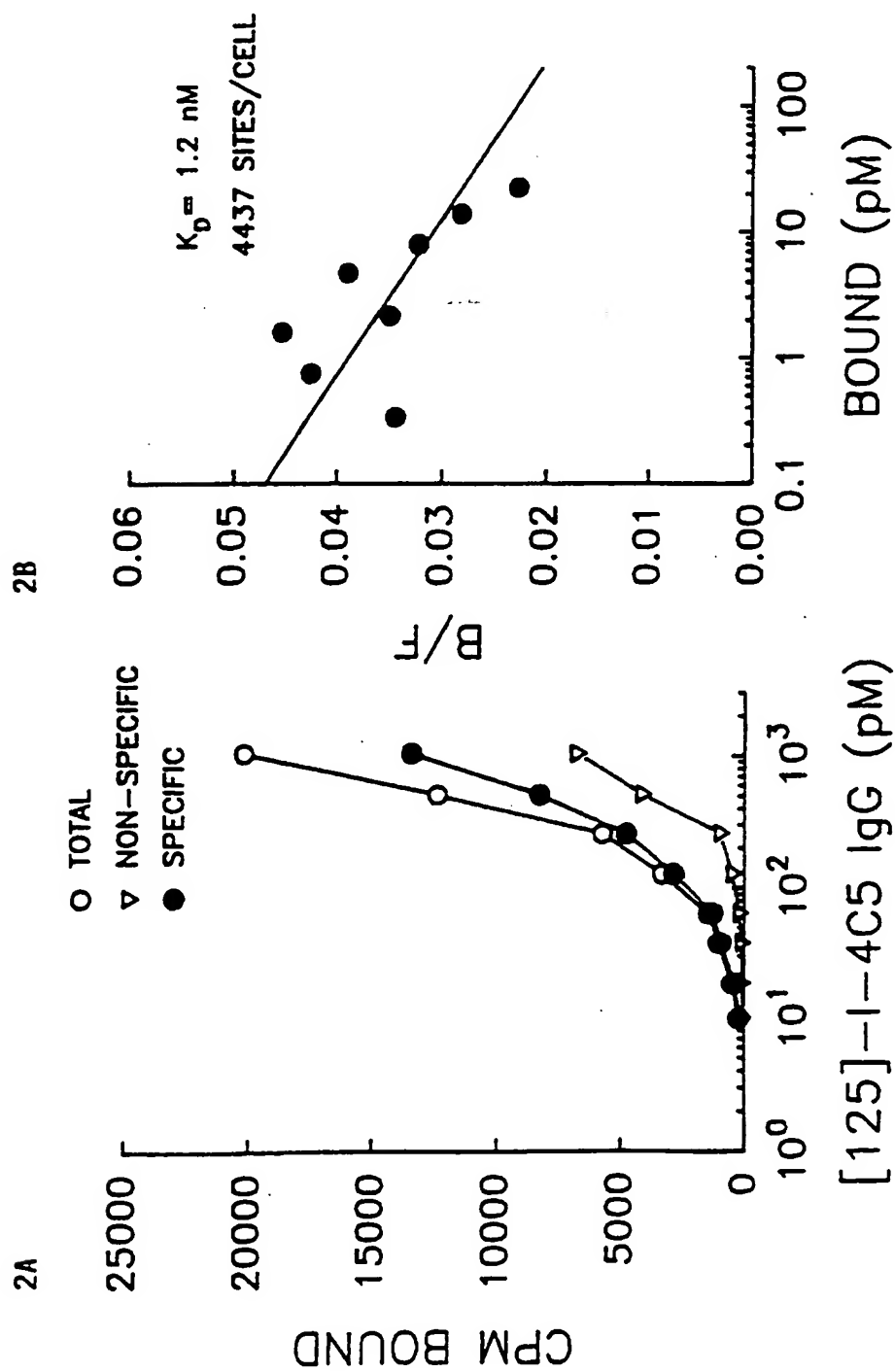


Fig. 3

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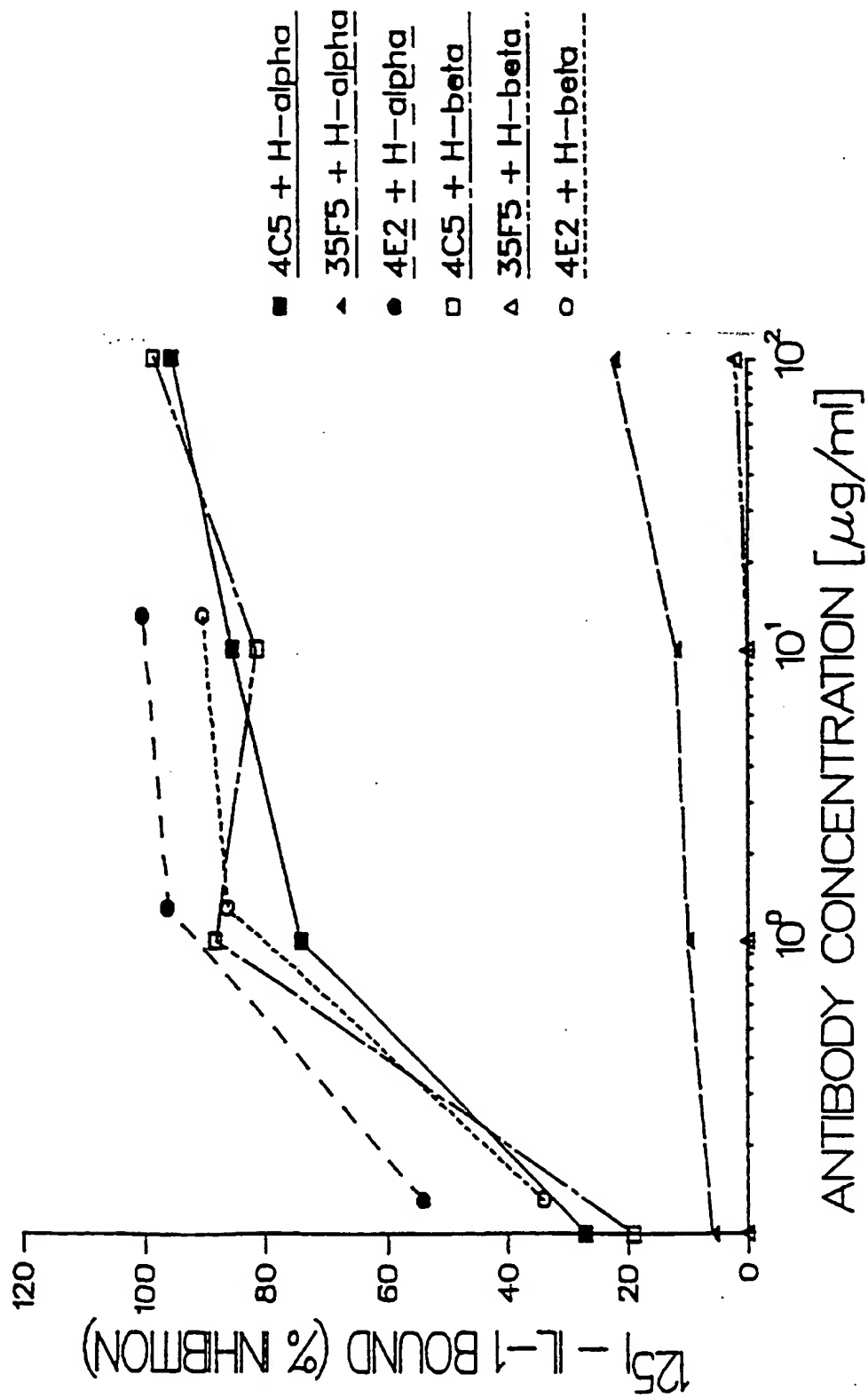
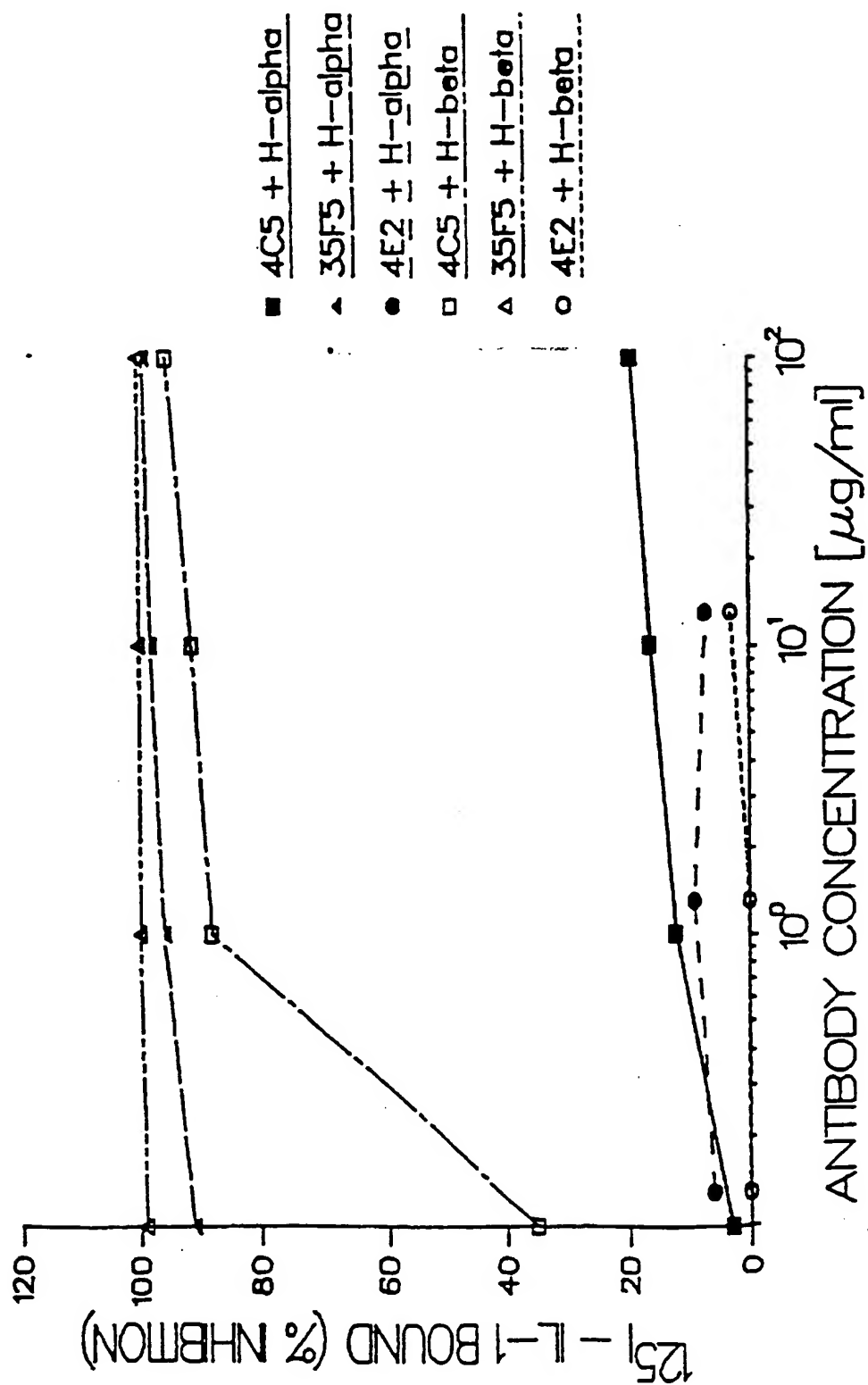


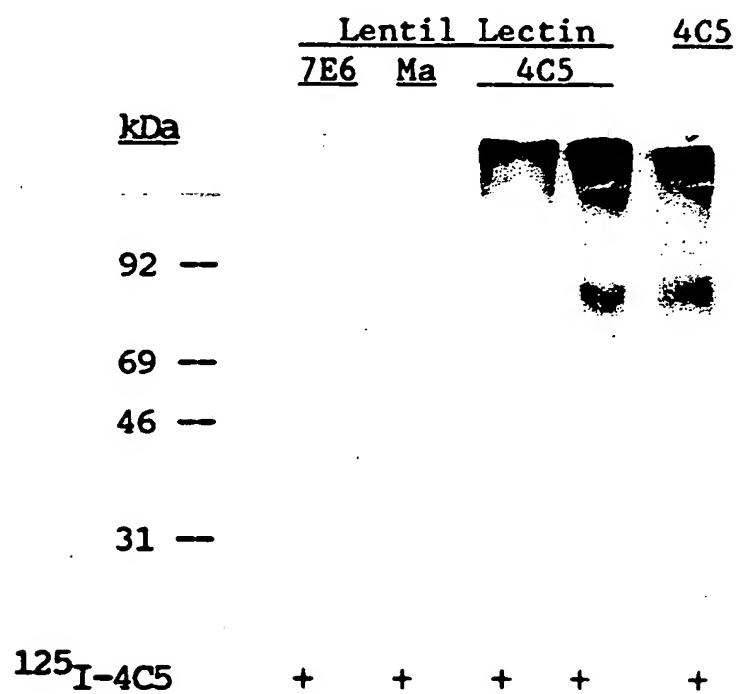
Fig. 4

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Fig. 5



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Fig. 6A

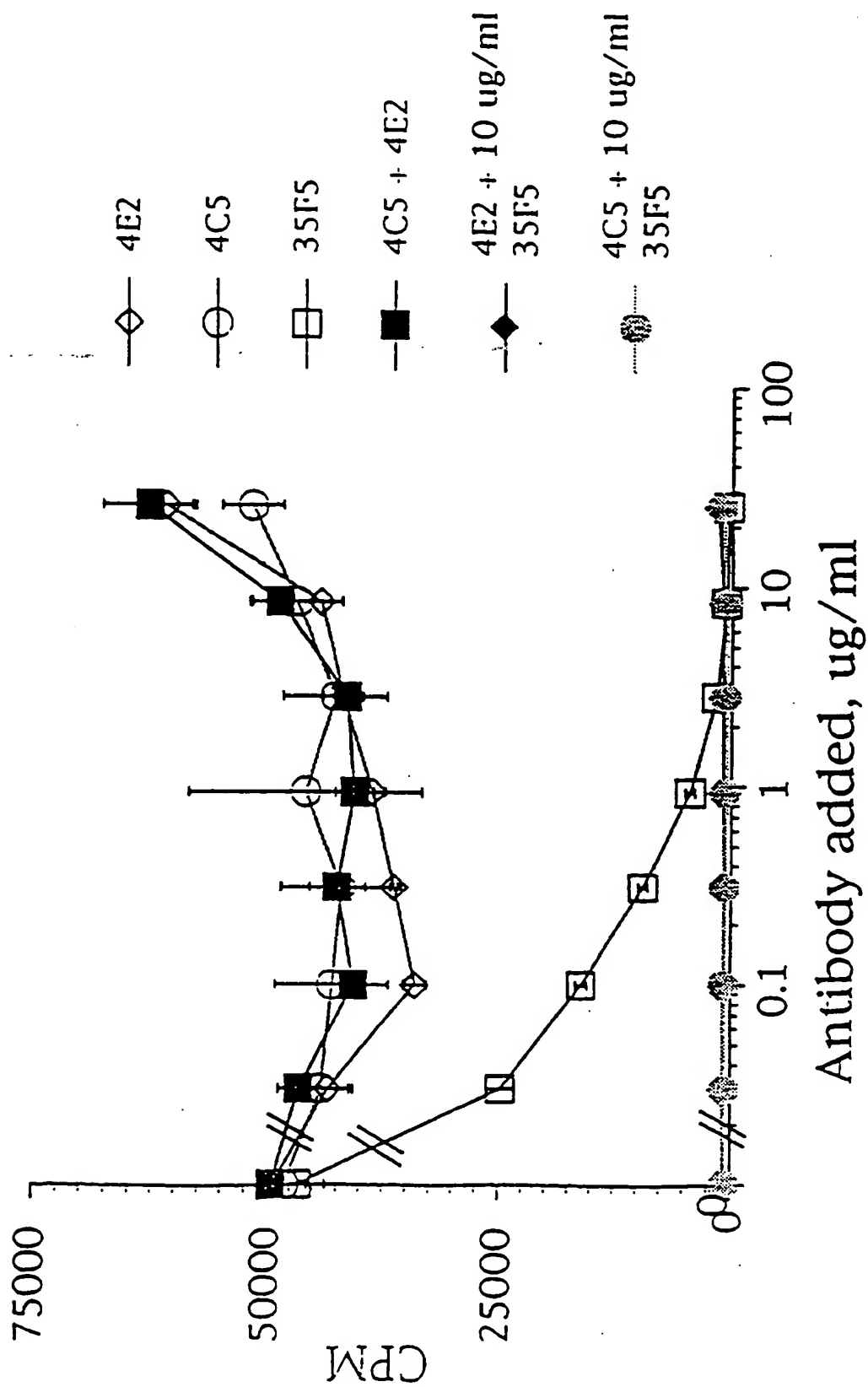
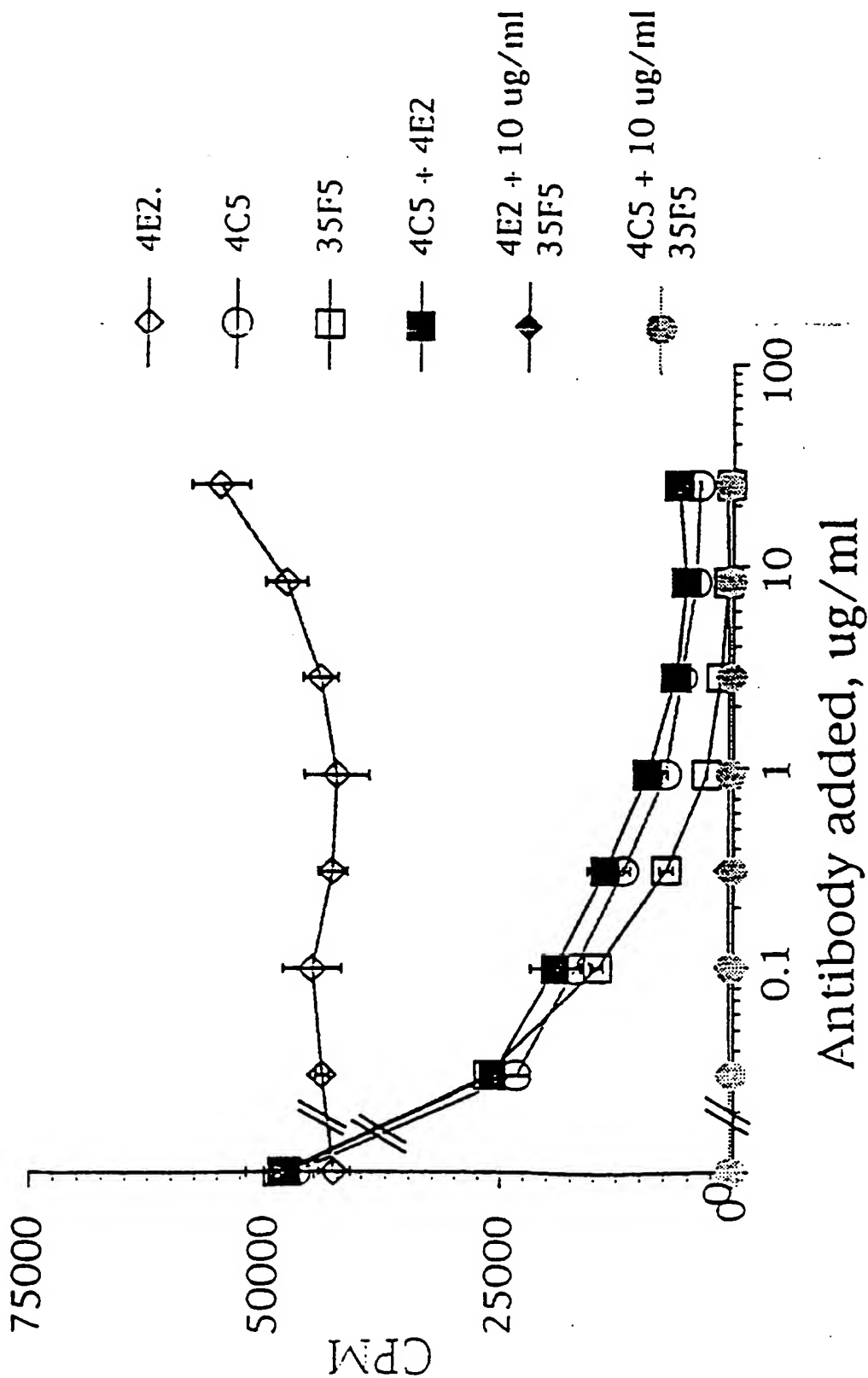


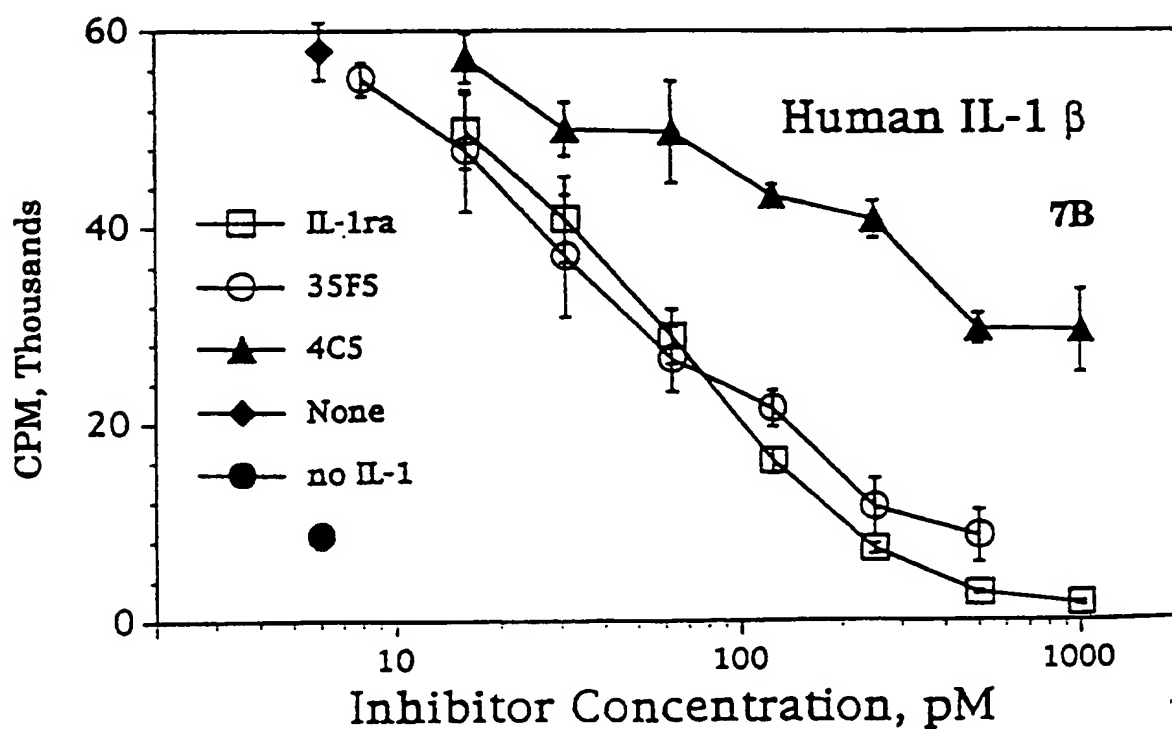
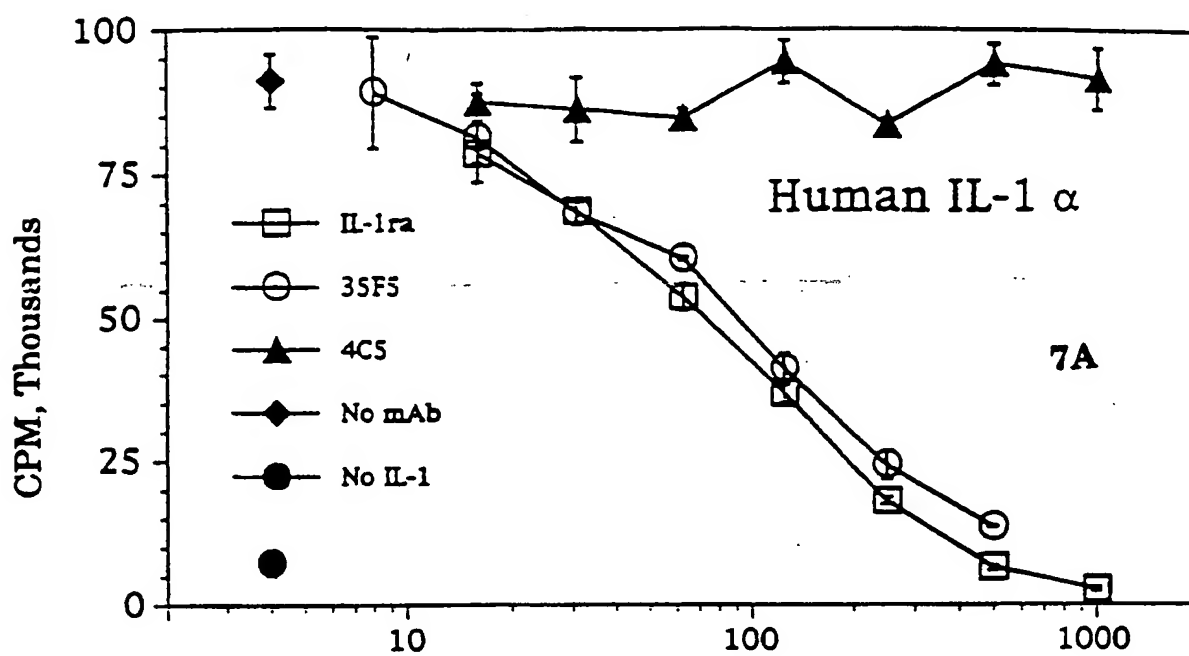
Fig. 6B

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Fig. 7



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Fig. 8

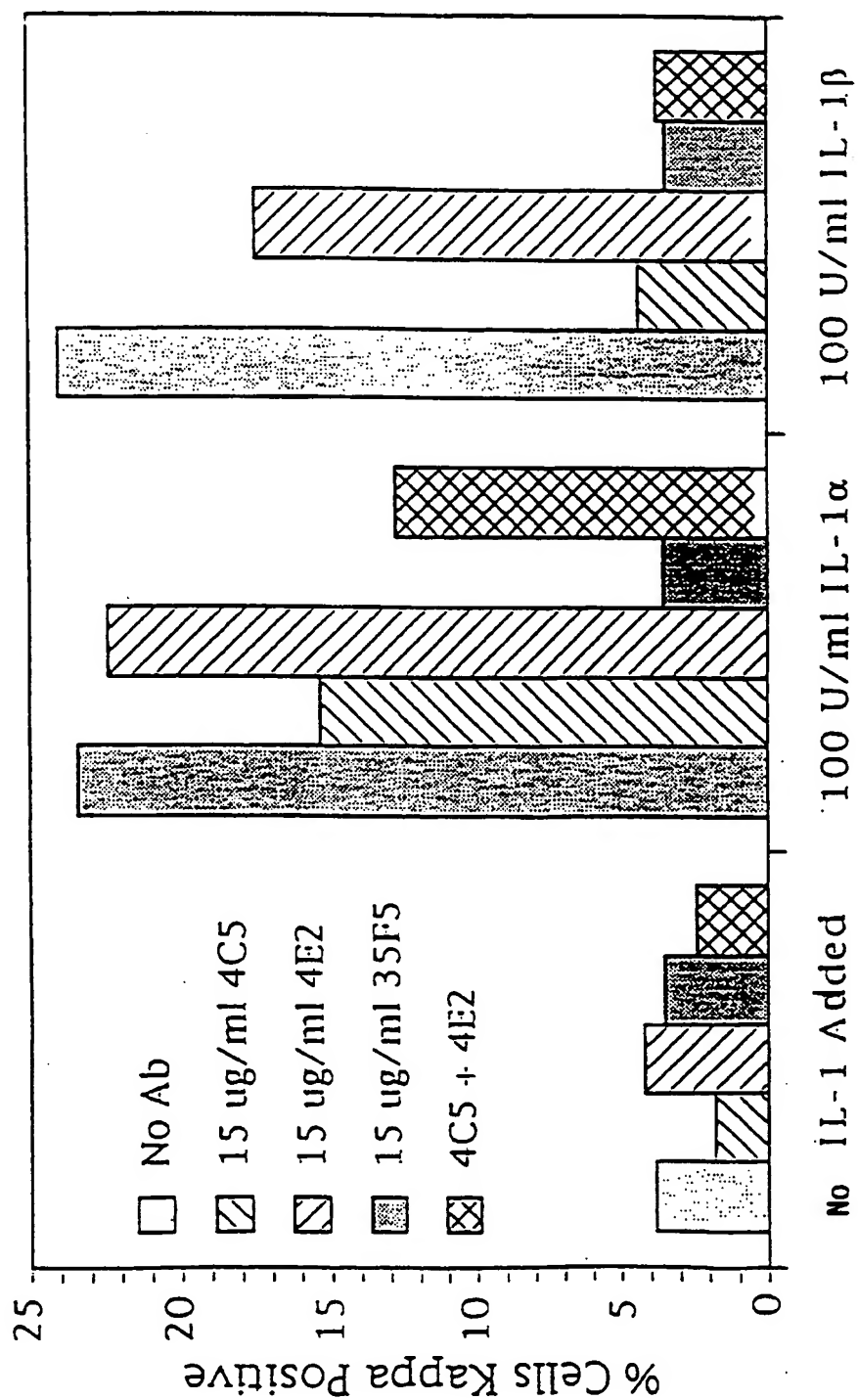
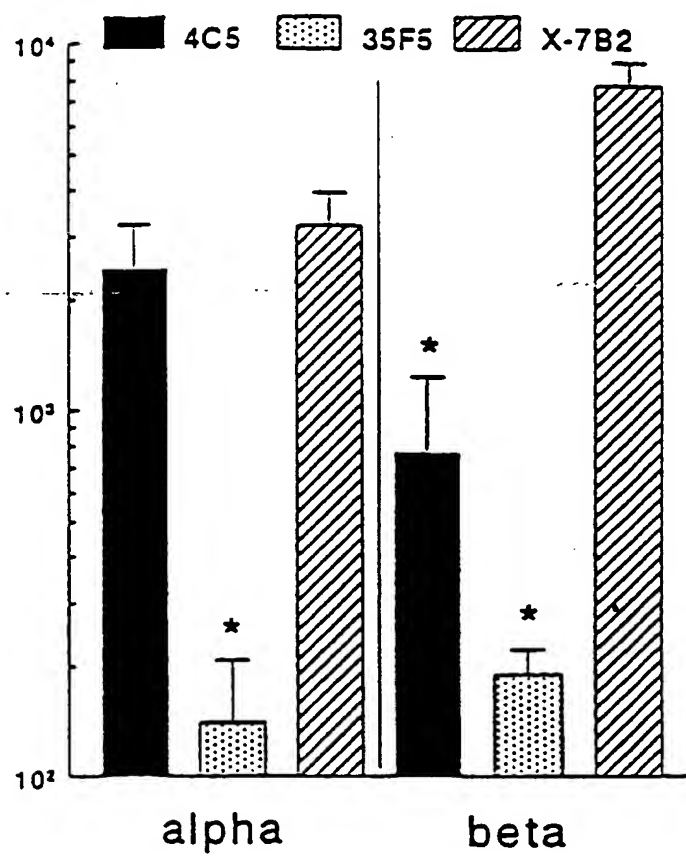


Fig. 9

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Fig. 10A

10	20	30	40	50	60	70
ATGGGACTTC	TGTGGTATTT	GATGAGTCTG	TCCTTCTATG	GGATCCTGCA	GAGTCATGCT	TCGGAGCGCT
TACCCCTGAAG	ACACCATAAA	CTACTCAGAC	AGGAAGATAC	CCTAGGACGT	CTCAGTACGA	AGCCTCGCGA
80	90	100	110	120	130	140
GTGATGACTG	GGGACTAGAT	ACCATGCGAC	AAATCCAAGT	GTTTGAAGAT	GAGCCGGCTC	GAATCAAGTG
CACTACTGAC	CCCTGATCTA	TGGTACGCTG	TTTAGGTTCA	CAAACCTTCTA	CTCGGCCGAG	CTTAGTTTCA
150	160	170	180	190	200	210
CCCCCTCTTT	GAACACTTCC	TGAAGTACAA	CTACAGCACT	GCCCATTCCT	CTGGCCTTAC	CCTGATCTGG
GGGGGAGAAA	CTTGTGAAGG	ACTTCATGTT	GATGTCGTGA	CGGGTAAGGA	GACCCGAATG	GGACTAGACC
220	230	240	250	260	270	280
TACTGGACCA	GGCAAGACCG	GGACCTGGAG	GAGCCCATTA	ACTTCCGCTT	CCCAGAGAAT	CGCATCAGTA
ATGACCTGGT	CCGTTCTGGC	CCTGGACCTC	CTCGGGTAAT	TGAAGGCGGA	GGGTCTCTTA	GCGTAGTCAT
290	300	310	320	330	340	350
AGGAGAAAGA	TGTGCTCTGG	TTCCGGCCCA	CCCTCCTCAA	TGACACGGGC	AATTACACCT	GCATGTTGAG
TCCTCTTTCT	ACACGAGACC	AAGGCCGGGT	GGGAGGAGTT	ACTGTGCCCC	TTAATGTGGA	CGTACAACCTC
360	370	380	390	400	410	420
GAACACAAC	TACTGCAGCA	AAGTTGCATT	TCCCCCTGAA	GTGTGTCAGA	AGGACAGCTG	TTTCAATTCT
CTTGTGTTGA	ATGACGTCGT	TTCAACGTAA	AGGGGACCTT	CAACAAGTCT	TCCTGTGCGAC	AAAGTTAAGA
430	440	450	460	470	480	490
GCCATGAGAT	TCCCAGTGCA	CAAGATGTAT	ATTGAACATG	GCAATTCATA	GATCACATGT	CCAAATGTAG
CGGTACTCTA	AGGGTACAGT	GTTCTACATA	TAACTTGTAC	CGTAAGTATT	CTAGTGTCAC	GTTTACATC
500	510	520	530	540	550	560
ACGGATACTT	TCCTTCCAGT	GTCAAACCAT	CGGTCACTTG	GTATAAGGGT	TGTACTGAAA	TAGTGGACTT
TGCCTATGAA	AGGAAGGTCA	CAGTTTGGTA	GCCAGTGAAC	CATATTCCCA	ACATGACTTT	ATCACCTGAA
570	580	590	600	610	620	630
TCATAATGTA	CTACCCGAGG	GCATGAACTT	GAGCTTTTTT	ATCCCCCTTG	TTTCAAATAA	CGGAAATTAC
AGTATTACAT	GATGGGCTCC	CGTACTTGAA	CTCGAAAAAG	TAGGGGAACC	AAAGTTTATT	GCCTTTAATG
640	650	660	670	680	690	700
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TGTACACACC	AATGTATAGG	ACTTTTGCCT	GCAGAGAAAG	TGGAGTGGTC	CTGACACTGA	CATTTCCACC
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TGGGCTCACC	AAAGGATGCA	TTGCCACCCC	AGATCTATTTC	TCCAAATGAC	CGTGTGTGCT	ATGAGAAAGA
ACCCGAGTGG	TTTCTACGT	AACGGTGGGG	TCTAGATAAG	AGGTTTACTG	GCACAACAGA	TACTCTTTCT
780	790	800	810	820	830	840
ACCAGGAGAG	GAACTGGTTA	TTCCCTGCAA	AGTCTATTTC	AGTTTCATTA	TGGACTCCCA	CAATGAGGTC
TGGTCCTCTC	CTTGACCAAT	AAGGGACGTT	TCAGATAAAG	TCAAAGTAAT	ACCTGAGGGT	GTTACTCCAG
850	860	870	880	890	900	910
TGGTGGACCA	TTGATGGAAA	GAAGCCTGAT	GACGTCACAG	TCGACATCAC	TATTAATGAA	AGTGTAAAGT
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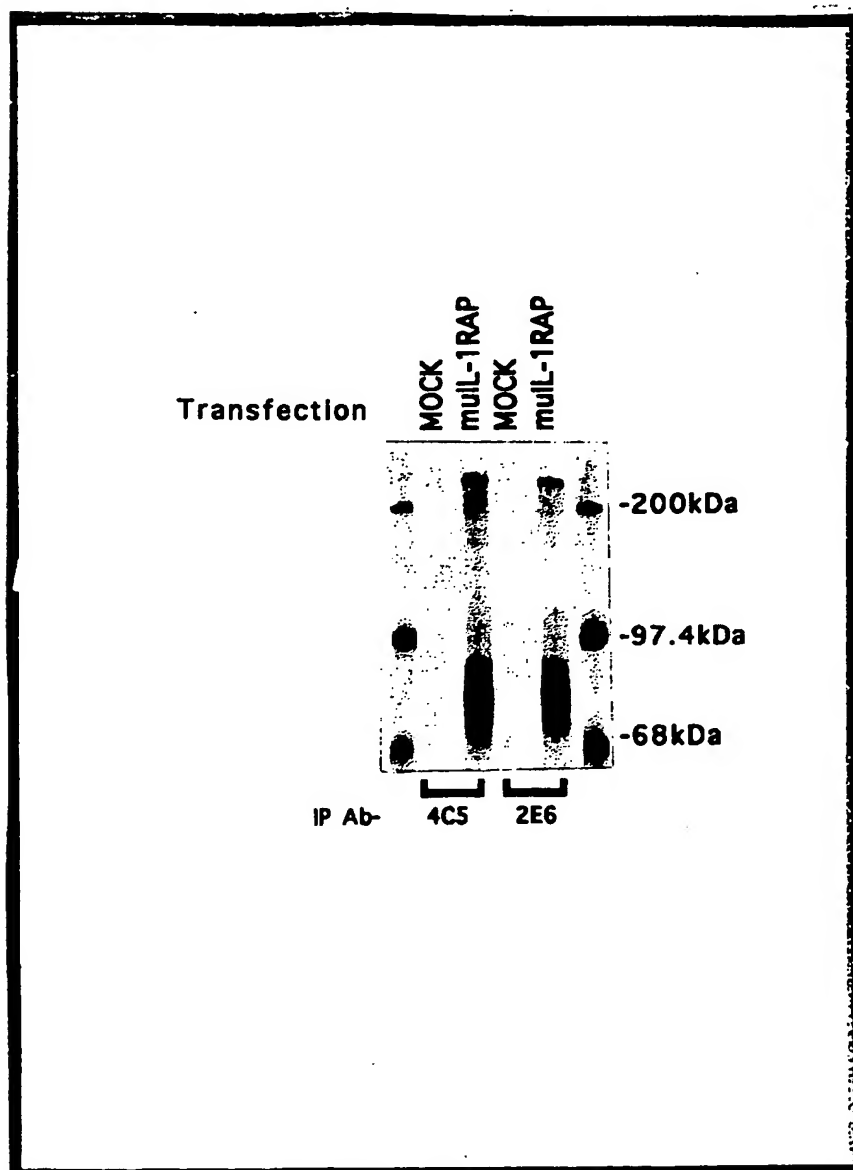
Fig. 10A cont.

920 ATTCTTCAAC TAAGAAGTTG	930 GGAAGATGAA CCTTCTACTT	940 ACAAGGACTC TGTTCCTGAG	950 AGATTTTGAG TCTAAAACCTC	960 CATCAAGAAA GTAGTTCCTT	970 GTCACCCCGG CAGTGGGGCC	980 AGGATCTCAG TCCTAGAGTC
990 GCGCAACTAT CGCGTTGATA	1000 GTCTGTCATG CAGACAGTAC	1010 CTCGAAATAC GAGCTTTTATG	1020 CAAAGGGGAA GTTTCCCTT	1030 GCTGAGCAGG CGACTCGTCC	1040 CTGCCAAGGT GACGGTTCCA	1050 GAAACAGAAA CTTTGTCTTT
1060 GTCATACCAC CAGTATGGTG	1070 CAAGGTACAC GTTCCATGTG	1080 AGTAGAACTC TCATCTTGAG	1090 GCCTGTGGTT CGGACACCAA	1100 TTGGAGCCAC AACCTCGGTG	1110 GGTCTTTCTG CCAGAAAGAC	1120 GTAGTGGTTC CATCACCAAG
1130 TCATTGTGGT AGTAACACCA	1140 TTACCATGTT AATGGTACAA	1150 TACTGGCTGG ATGACCGACC	1160 AGATGGTCCT TCTACCAGGA	1170 CTTTTACCGA GAAAATGGCT	1180 GCTCACTTTG CGAGTGAAAC	1190 GAACAGATGA CTTGTCTACT
1200 ACAATTCTT TTGTTAAGAA	1210 GATGGAAAGG CTACCTTTCC	1220 AGTATGATAT TCATACTATA	1230 TTATGTTTCC AATACAAAGG	1240 TATGCAAGAA ATACGTTCTT	1250 ATGTGGAAGA TACACCTTCT	1260 AGAGGAATTT TCTCCTTAA
1270 GTGCTGCTGA CACGACGACT	1280 CGCTGCGTGG GCGACGCACC	1290 AGTTTTGGAG TCAAAACCTC	1300 AATGAGTTTG TTACTCAAAC	1310 GATACAAGCT CTATGTTCTGA	1320 GTGCATCTTC CACGTAGAAG	1330 GACAGAGACA CTGTCTCTGT
1340 GCCTGCCTGG CGGACGGACC	1350 GGGAATTGTC CCCTTAACAG	1360 ACAGATGAGA TGTCTACTCT	1370 CCCTGAGCTT GGGACTCGAA	1380 CATTCAGAAA GTAAGTCTTT	1390 AGCAGACGAC TCGTCTGCTG	1400 TCCTGGTTGT AGGACCAACA
1410 CCTAAGTCCC GGATTCAAGG	1420 AACTACGTGC TTGATGCACG	1430 TCCAGGGAAC AGGTCCCTTG	1440 ACAAGCCCTC TGTTCCGGAG	1450 CTGGAGCTCA GACCTCGAGT	1460 AGGCTGGCCT TCCGACCGGA	1470 AGAAAATATG TCTTTTATAC
1480 GCCTCCCGGG CGGAGGGCCC	1490 GCAACATCAA CGTTGTAGTT	1500 CGTCATTTTA GCAGTAAAAT	1510 GTGCAGTACA CACGTCAATG	1520 AAGCTGTGAA TTCGACACTT	1530 GGACATGAAG CCTGTACTTC	1540 GTGAAAGAGC CACTTTCTCG
1550 TGAAGCGGGC ACTTCGCCCG	1560 TAAGACGGTG ATTCTGCCAC	1570 CTCACGGTCA GAGTGCCAGT	1580 TTAAATGGAA AATTTACCTT	1590 AGGAGAGAAA TCCTCTCTTT	1600 TCCAAGTATC AGGTTTCATAG	1610 CTCAGGGCAG GAGTCCCCTC
1620 GTTCTGGAAG CAAGACCTTC	1630 CAGTTGCAGG GTCAACGTCC	1640 TGGCCATGCC ACCGGTACGG	1650 AGTGAAGAAG TCACTTCTTC	1660 AGTCCAGGT TCAGGGTCCA	1670 GGTCTAGCAA CCAGATCGTT	1680 TGACAAGCAG ACTGTTCTGC
1690 GGTCTCTCCT CCAGAGAGGA	1700 ACTCATCCCT TGAGTAGGGA	1710 GAAAAACGTA CTTTTTGTCAT	TGA ACT			

-10	-1	10	20	30	40	50
MGLLWYLSL	SFYGILQSHA	SERCDDWGLD	TMRQIQVFED	EPARIKCPLF	EHFLKYNYST	AHSSGLTLIW
60	70	80	90	100	110	120
YWTRQDRDLE	EPINFRLPEN	RISKEKDVW	FRPTLLNDTG	NYTCMLRNTT	YCSKVAPPLE	VVQKDSCFNS
130	140	150	160	170	180	190
AMRFPVHKMY	IEHGIHKITC	PNVDGYFPSS	VKPSVTWYKG	CTETVDFHNV	LPEGMNLSFF	IPLVSNNNGNY
200	210	220	230	240	250	260
TCVVTPENG	RLFHLTRTVT	VKVVGSPKDA	LPPQIYSPND	RVVYEKEPGE	ELVIPCKVYP	SFIMDSHNEV
270	280	290	300	310	320	330
WWTIDGKKPD	DVTVDITINE	SVSYSTEDE	TRTQILSIKK	VTPEDLRNRY	VCHARNTKGE	AEQAAKVKQK
340	350	360	370	380	390	400
VIPPRYTVEL	ACGFGATVFL	VVVLIVVYHV	YWLEMVLFYR	AHFGTDEIIL	DGKEYDIYVS	YARNVEEEEF
410	420	430	440	450	460	470
VLLTLRGVLE	NEFGYKLCIF	DRDSLPGGIV	TDETLSFIQK	SRRLVVLSP	NYVLQGTQAL	LELKAGLENM
480	490	500	510	520	530	540
ASRGNINVL	VQYKAVKDMK	VKELKRAKTV	LTVIKWKGEK	SKYPQGRFWK	QLQVAMPVKK	SPRWSSNDKQ
550						
GLSYSSLKRV						

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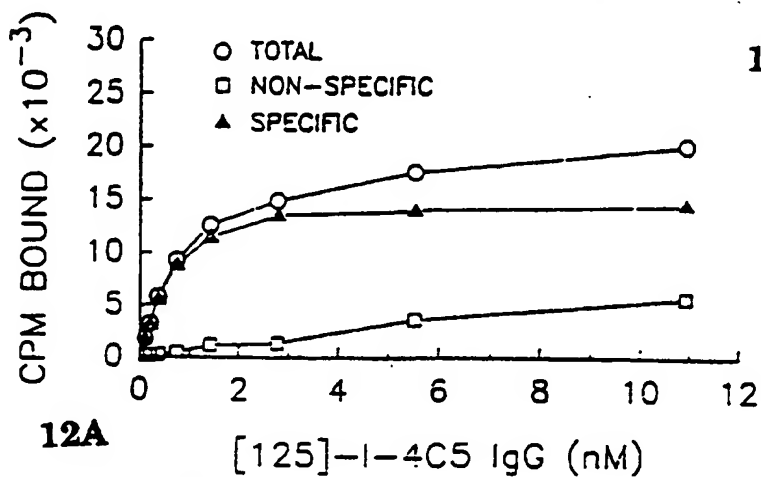
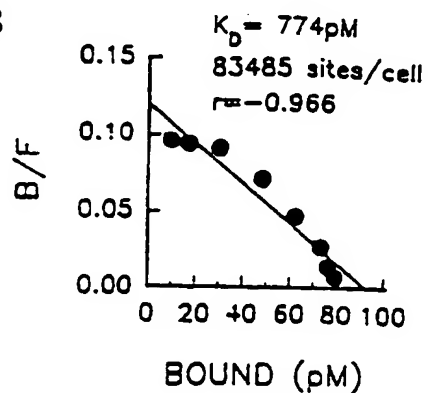
Fig. 11



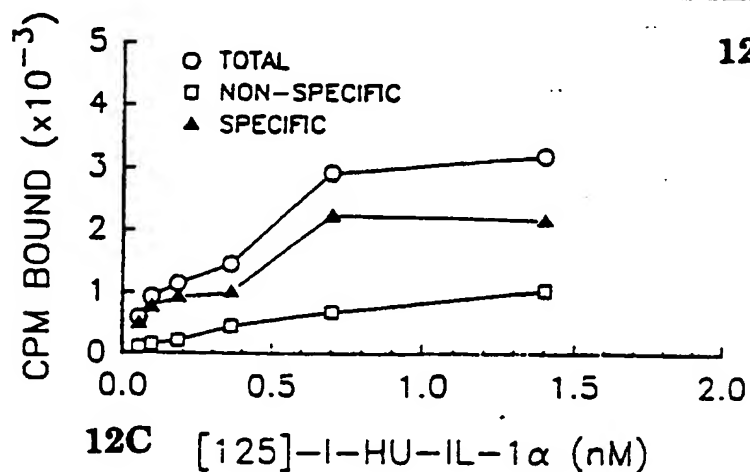
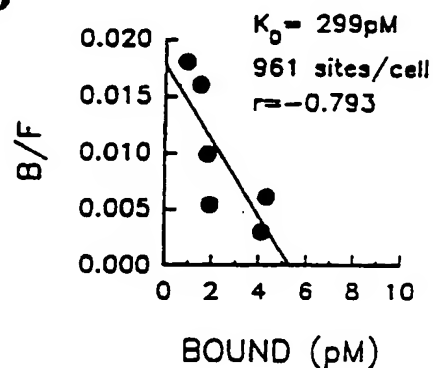
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[125]-I-4C5 IgG BINDING TO COS(AcP) CELLS

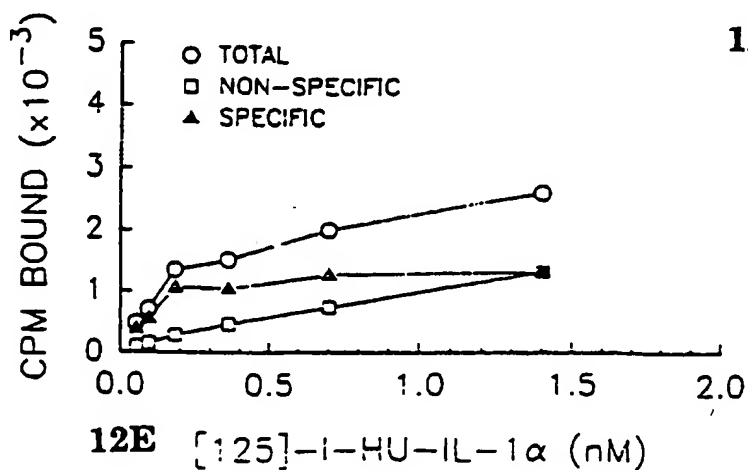
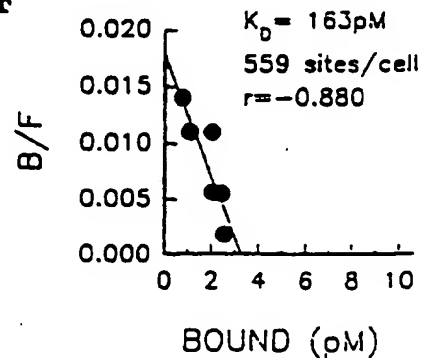
Fig. 12

**12B**

[125]-I-HU-IL-1 α BINDING TO COS(AcP) CELLS

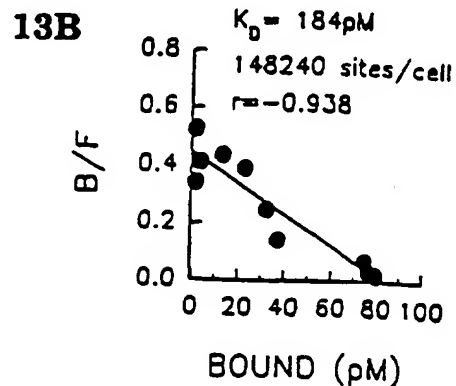
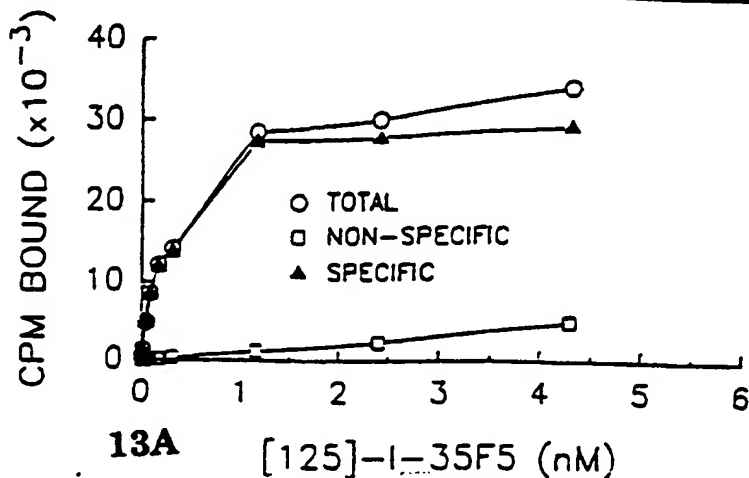
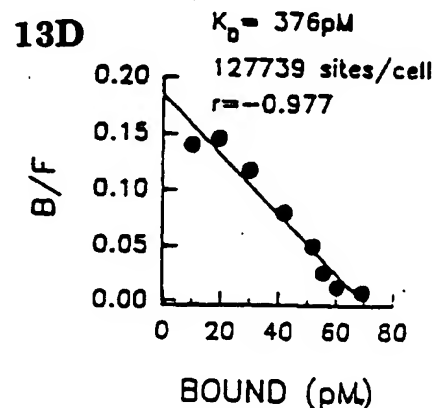
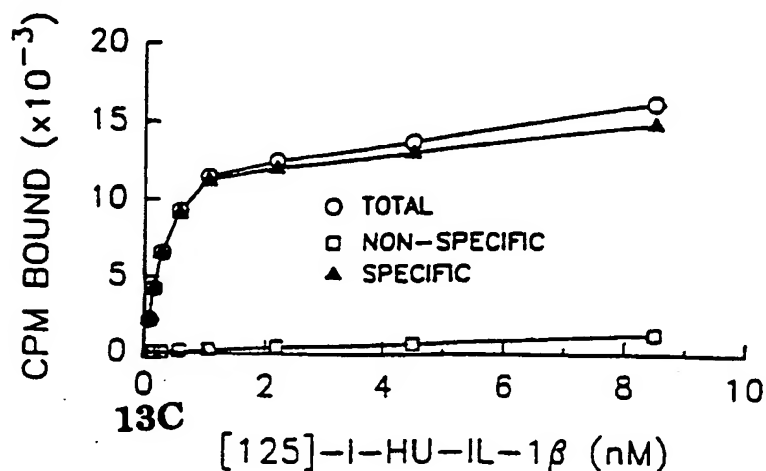
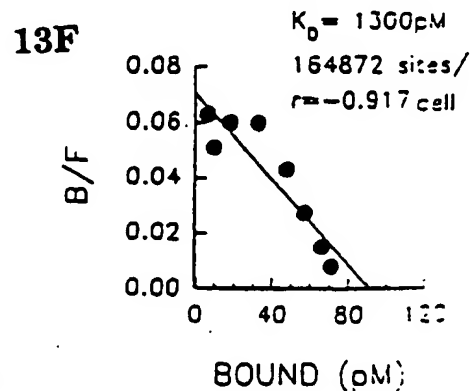
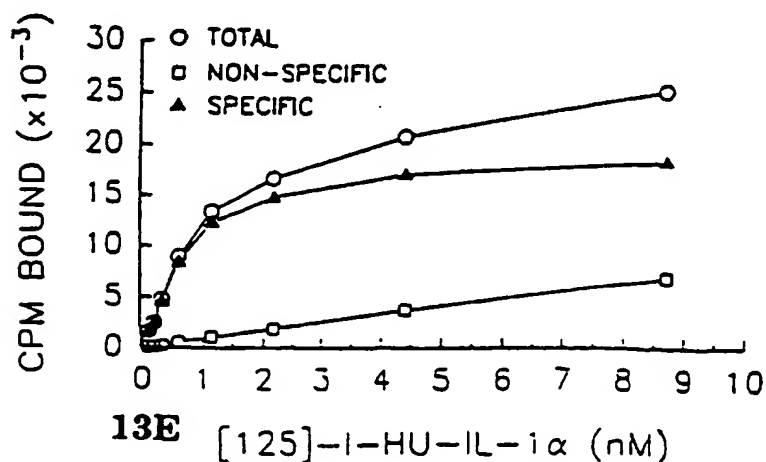
**12D**

[125]-I-HU-IL-1 α BINDING TO COS(PEF-BOS) CELLS

**12F**

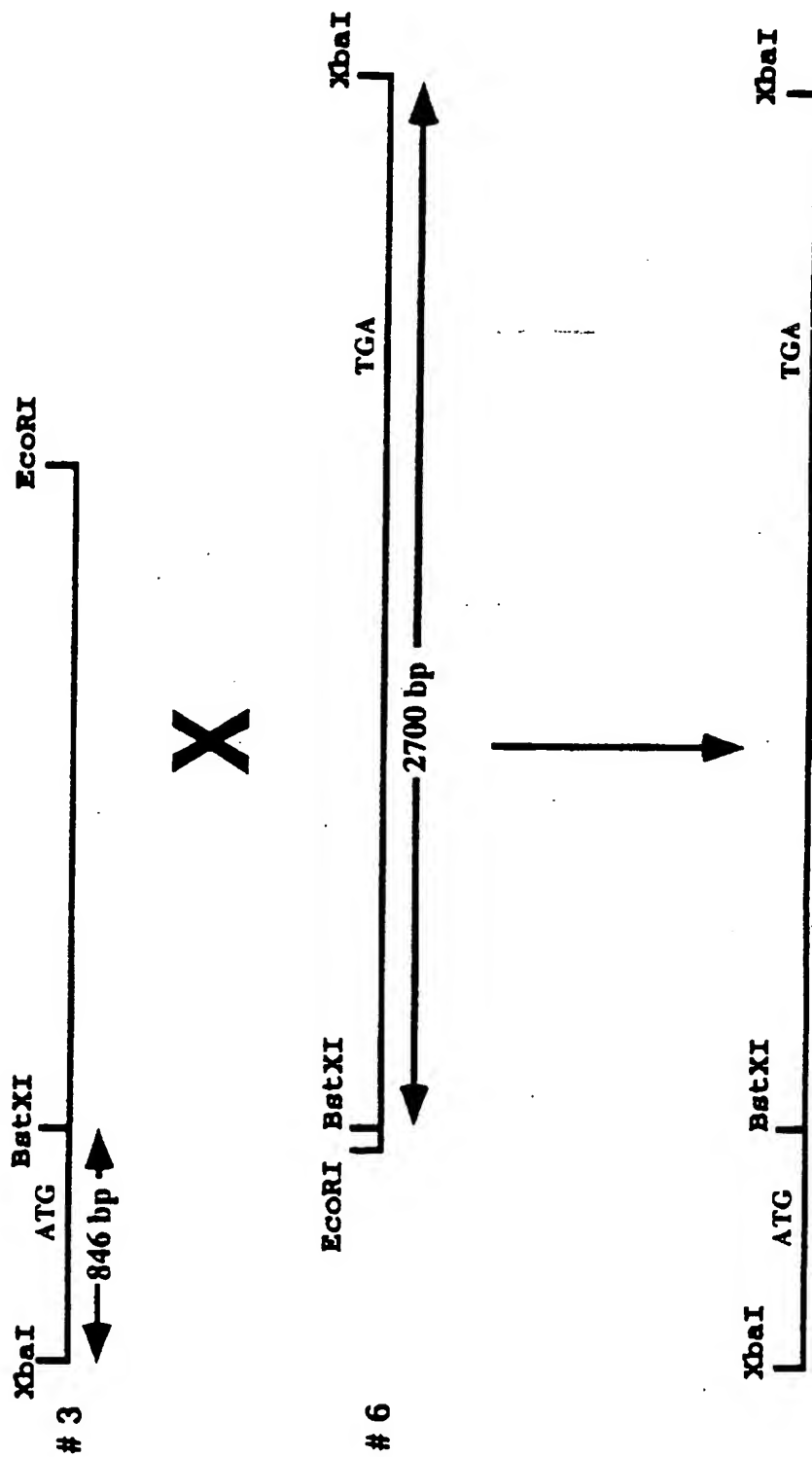
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Fig. 13

[125]-I-35F5 IgG BINDING TO COS(MU-IL-1R) CELLS[125]-I-HU-IL-1 β BINDING TO COS(MU-IL-1R) CELLS[125]-I-HU-IL-1 α BINDING TO COS(MU-IL-1R) CELLS

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Fig. 14



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Fig. 15

10	20	30	40	50	60	70
ATGACACTTC	TGTGGTGTGT	AGTGAGTCTC	TACTTTTATG	GAATCCTGCA	AAGTGATGCC	TCAGAACGCT
TACTGTGAAG	ACACCACACA	TCACTCAGAG	ATGAAAATAC	CTTAGGACGT	TTCCTACGG	AGTCTTGCGA
80	90	100	110	120	130	140
GCGATGACTG	GGGACTAGAC	ACCATGAGGC	AAATCCAAGT	GTTTGAAGAT	GAGCCAGCTC	GCATCAAGTG
CGCTACTGAC	CCCTGATCTG	TGGTACTCCG	TTTAGGTTCA	CAAACCTTCTA	CTCGGTCGAG	CGTAGTTTCA
150	160	170	180	190	200	210
CCCACTCTTT	GAACACTTCT	TGAAATTCAA	CTACAGCACA	GCCCATTCAG	CTGGCCTTAC	TCTGATCTGG
GGGTGAGAAA	CTTGTAAGA	ACTTTAAGTT	GATGTCGTGT	CGGGTAAGTC	GACCGGAATG	AGACTAGACC
220	230	240	250	260	270	280
TATTGGACTA	GGCAGGACCG	GGACCTTGAG	GAGCCAATTA	ACTTCCGCCT	CCCCGAGAAC	CGCATTAGTA
ATAACCTGAT	CCGTCTCTGGC	CCTGGAATC	CTCGGTAAAT	TGAAGGCGGA	GGGGCTCTTG	GCGTAATCAT
290	300	310	320	330	340	350
AGGAGAAAGA	TGTGCTGTGG	TTCCGGCCCA	CTCTCCTCAA	TGACACTGGC	AACTATACCT	GCATGTTAAG
TCCTCTTTCT	ACACGACACC	AAGGCCGGGT	GAGAGGAGTT	ACTGTGACCG	TTGATATGGA	CGTACAATTC
360	370	380	390	400	410	420
GAACACTACA	TATTGCAGCA	AAGTTGCATT	TCCCTTGGAA	GTTGTTCAAA	AAGACAGCTG	TTTCAATTCC
CTTGTGATGT	ATAACGTCGT	TTCAACGTAA	AGGGAACCTT	CAACAAGTTT	TTCTGTGAC	AAAGTTAAGG
430	440	450	460	470	480	490
CCCATGAAAC	TCCCAGTGCA	TAACTGTAT	ATAGAATATG	GCATTCAGAG	GATCACTTGT	CCAAATGTAG
GGGTACTTTG	AGGGTCACGT	ATTTGACATA	TATCTTATAC	CGTAAGTCTC	CTAGTGAACA	GGTTTACATC
500	510	520	530	540	550	560
ATGGATATTT	TCCTTCCAGT	GTCAAACCGA	CTATCACTTG	GATATATGGC	TGTTATAAAA	TACAGAATTT
TACCTATAAA	AGGAAGGTCA	CAGTTTGGCT	GATAGTGAAC	CATATACCCG	ACAATATTTT	ATGTCTTAAA
570	580	590	600	610	620	630
TAATAATGTA	ATACCCGAAG	GTATGAACTT	GAGTTTCCTC	ATTGCCTTAA	TTTCAAATAA	TGGAAATTAC
ATTATTACAT	TATGGGCTTC	CATACTTGAA	CTCAAAGGAG	TAACGGAATT	AAAGTTTATT	ACCTTTAATG
640	650	660	670	680	690	700
ACATGTGTTG	TTACATATCC	AGAAAATGGA	CGTACGTTTC	ATCTCACCAG	GACTCTGACT	GTAAAGGTAG
TGTACACAAC	AATGTATAGG	TCTTTTACCT	GCATGCAAAG	TAGAGTGGTC	CTGAGACTGA	CATTTCCATC
710	720	730	740	750	760	770
TAGGCTCTCC	AAAAAATGCA	GTGCCCCCTG	TGATCCATTG	ACCTAATGAT	CATGTGGTCT	ATGAGAAAAGA
ATCCGAGAGG	TTTTTTACGT	CACGGGGGAC	ACTAGGTAAG	TGGATTACTA	GTACACCAGA	TACTCTTTCT
780	790	800	810	820	830	840
ACCAGGAGAG	GAGCTACTCA	TTCCCTGTAC	GGTCTATTTT	AGTTTCTGA	TGGATTCTCG	CAATGAGGTT
TGGTCCTCTC	CTCGATGAGT	AAGGGACATG	CCAGATAAAA	TCAAAAGACT	ACCTAAGAGC	GTTACTCCAA
850	860	870	880	890	900	910
TGGTGGACCA	TTGATGGAAA	AAAACCTGAT	GACATCACTA	TTGATGTCAC	CATTAAACGAA	AGTATAAGTC
ACCACCTGGT	AACCTACCTT	TTTTGGACTA	CTGTAGTGAT	AACTACAGTG	GTAATTGCTT	TCATATTGAG

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Fig. 15 cont.

920	930	940	950	960	970	980
ATAGTAGAAC	AGAAGATGAA	ACAAGAACTC	AGATTTTGAG	CATCAAGAAA	GTTACCTCTG	AGGATCTCAA
TATCATCTTG	TCTTCTACTT	TGTTCTTGAG	TCTAAAACTC	GTAGTTCCTT	CAATGGAGAC	TCCTAGAGTT
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CGCGTCGATA	CAGACAGTAC	GATCTTCACG	GTTTCCGCTT	CAACGGTTTC	GTCGGTTCCA	CTGCGTCTTT
1060	1070	1080	1090	1100	1110	1120
GTGCCAGCTC	CAAGATACAC	AGTGGAACTG	GCTTGTTGGT	TTGGAGCCAC	AGTCCTGCTA	GTGGTGATTC
CACGGTCGAG	GTTCTATGTG	TCACCTTGAC	CGAACACCAA	AACCTCGGTG	TCAGGACGAT	CACCACTAAG
1130	1140	1150	1160	1170	1180	1190
TCATTGTTGT	TTACCATGTT	TACTGGCTAG	AGATGGTCCT	ATTTTACCGG	GCTCATTTTG	GAACAGATGA
AGTAACAACA	AATGGTACAA	ATGACCGATC	TCTACCAGGA	TAAAATGGCC	CGAGTAAAC	CTTGTCTACT
1200	1210	1220	1230	1240	1250	1260
AACCATTTTA	GATGGAAAAG	AGTATGATAT	TTATGTATCC	TATGCAAGGA	ATGCGGAAGA	AGAAGAATTT
TTGGTAAAAT	CTACCTTTTC	TCATACTATA	AATACATAGG	ATACGTTCTT	TACGCCTTCT	TCTTCTTAAA
1270	1280	1290	1300	1310	1320	1330
GTTTTACTGA	CCCTCCGTGG	AGTTTTGGAG	AATGAATTTG	GATACAAGCT	GTGCATCTTT	GACCGAGACA
CAAAATGACT	GGGAGGCACC	TCAAAACCTC	TTACTTAAAC	CTATGTTCGA	CACGTAGAAA	CTGGCTCTGT
1340	1350	1360	1370	1380	1390	1400
GTCTGCCTGG	GGGAATTGTC	ACAGATGAGA	CTTTGAGCTT	CATTCAGAAA	AGCAGACGCC	TCCTGGTTGT
CAGACGGACC	CCCTTAACAG	TGTCTACTCT	GAAACTCGAA	GTAAGTCTTT	TCGTCTGCGG	AGGACCAACA
1410	1420	1430	1440	1450	1460	1470
TCTAAGCCCC	AACTACGTGC	TCCAGGGAAC	CCAAGCCCTC	CTGGAGCTCA	AGGCTGGCCT	AGAAAATATG
AGATTGCGGG	TTGATGCACG	AGGTCCCTTG	GGTTCGGGAG	GACCTCGAGT	TCCGACCGGA	TCTTTTATAC
1480	1490	1500	1510	1520	1530	1540
GGCTCTCGGG	GCAACATCAA	CGTCATTTTA	GTACAGTACA	AAGCTGTGAA	GGAAACGAAG	GTGAAAGAGC
CCGAGAGCCC	CGTTGTAGTT	GCAGTAAAAAT	CATGTCATGT	TTGACACTT	CCTTTGCTTC	CACTTTCTCG
1550	1560	1570	1580	1590	1600	1610
TGAAGAGGGC	TAAGACGGTG	CTCACGGTCA	TTAAATGGAA	AGGGGAAAAA	TCCAAGTATC	CACAGGGCAG
ACTTCTCCCG	ATTCTGCCAC	GAGTGCCAGT	AATTTACCTT	TCCCCTTTT	AGGTTTCATAG	GTGTCCCGTC
1620	1630	1640	1650	1660	1670	1680
GTTCTGGAAG	CAGCTGCAGG	TGGCCATGCC	AGTGAAGAAA	AGTCCCAGGC	GGTCTAGCAG	TGATGAGCAG
CAAGACCTTC	GTCGACGTCC	ACCGGTACGG	TCACTTCTTT	TCAGGGTCCG	CCAGATCGTC	ACTACTCGTC
1690	1700	1710				
GGCCTCTCGT	ATTCATCTTT	GAAAAATGTA	TGA			
CCGGAGAGCA	TAAGTAGAAA	CTTTTACAT	ACT			

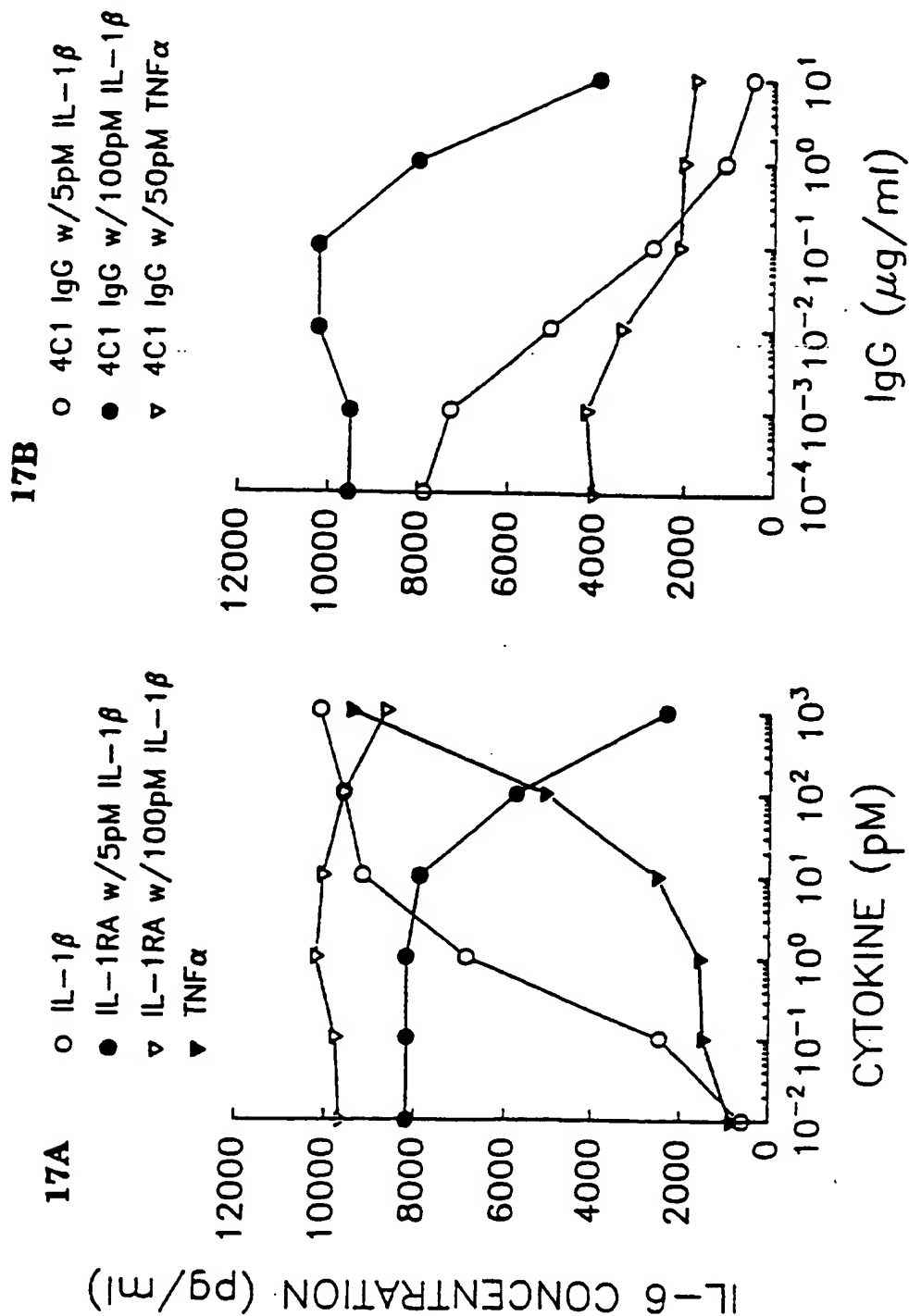
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Fig. 16

-20	-10	-1	1	10	20	30	40	50
MTLLNCVVS	YFYGILQSDA	SERCDDWG	LDTMRQIQVF	ED	EPARIKCP	LF	ZHFLKFN	YST AHSAGLTLIW
60	70	80	90	100	110	120		
YWTRQDRDLE	EPINFRLPEN	RISKEKDVLW	FRPTLLNDTG	NYTCMLRN	TT	YCSKVAFPLE	VVQKDS	CFNS
130	140	150	160	170	180	190		
PMKLPVHKLY	IEYGIQRITC	PNVDGYFPSS	VKPTITWYMG	CYKIQNFNNV	IPEGMNL	SFL	LALISN	NGNY
200	210	220	230	240	250	260		
TCVVITYPENG	RTFHLTRILT	VKVVGSPKNA	VPPVIHSPND	HVVYEKEPGE	ELLIPCTVYF	SFLMDSRNEV		
270	280	290	300	310	320	330		
NWTIDGKKPD	DITIDVTINE	SISHSRTEDE	TRTQILSIKK	VTSEDLKRSY	VCHARSAKGE	VAKAAKVTQK		
340	350	360	370	380	390	400		
VPAPRYTVEL	ACGFGATVLL	VVILIVVYHV	YWLEMVLFYR	AHFGTDETIL	DGKEYDIYVS	YARNAEEEEZF		
410	420	430	440	450	460	470		
VLLTLRGVLE	NEFGYKLCIF	DRDSLPGGIV	TDETLSFIQK	SRLLVVLSP	NYVLQGTQAL	LELKAGLENM		
480	490	500	510	520	530	540		
GSRGNINVIL	VOYKAVKETK	VKELKRAKTV	LTVIKWKGEK	SKYPQGRFWK	QLQVAMPVKK	SPRASSSDEQ		
550								
GLSYSSLKQV								

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Fig. 17



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Fig. 18

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10      20      30      40      50      60      70
ATGACACTTC TGTGGTGTGT AGTGAGTCTC TACTTTTATG GAATCCTGCA AAGTGATGCC TCAGAAGCGT
TACTGTGAAG ACACCACACA TCACTCAGAG ATGAAAATAC CTTAGGACGT TTCACTACGG AGTCTTGGCA

80      90      100     110     120     130     140
GGCATGACTG GGGACTAGAC ACCATGAGGC AAATCCAAGT GTTTGAAGAT GAGCCAGCTC GCATCAAGTG
CGCTACTGAC CCCTGATCTG TGGTACTCCG TTTAGGTTCA CAAACTTCTA CTCGGTCCGAG CGTAGTTTAC

150     160     170     180     190     200     210
CCCACTCTTT GAACACTTCT TGAAATTCAA CTACAGCACA GCCCAATCAG CTGGCCTTAC TCTGATCTGG
GGGTGAGAAA CTTGTGAAGA ACTTTAAGTT GATGTCGTGT CGGGTAAAGTC GACCGGAATG AGACTAGACC

220     230     240     250     260     270     280
TATTGGACTA GGCAGGACCG GGACCTTGAG GAGCCAATTA AGTTCCGCCT CCCCAGAGAAC CGCATTAGTA
ATAACCTGAT CCGTCTCTGC CCTGGAATC CTGCGTTAAT TGAAGGCGGA GGGGCTCTTG CGGTAAATCAT

290     300     310     320     330     340     350
AGGAGAAAGA TGTGCTGTGG TTCCGGCCCA CTCTCCTCAA TGACACTGGC AACTATACCT GCATGTTAAG
TCCTCTTTCT ACACGACACC AAGGCCGGGT GAGAGGAGTT ACTGTGACCG TTGATATGGA CGTACAATTC

360     370     380     390     400     410     420
GAACACTACA TATTGCAGCA AAGTTGCATT TCCCTTGGAA GTTGTTCAAA AAGACAGCTG TTTCAATTCC
CTTGTGATGT ATAACGTCGT TTCAACGTAA AGGGAACCTT CAACAAGTTT TTCTGTCCGAC AAAGTTAAGG

430     440     450     460     470     480     490
CCCATGAAC TCCCACTGCA TAAACTGTAT ATAGAATATG GCATTGAGAG GATCACTTGT CCAAATGTAG
GGGTACTTTG AGGGTCACGT ATTTGACATA TATCTTATAC CGTAAGTCTC CTAGTGAACA GGTTTACATC

500     510     520     530     540     550     560
ATGGATATTT TCCTTCCAGT GTCAAACCGA CTATCACTTG GTATATGGGC TGTATAAAAA TACAGAAATT
TACCTATAAA AGGAAGGTCA CAGTTTGGCT GATAGTGAAC CATATACCCG ACAATATTTT ATGTCTTAAA

570     580     590     600     610     620     630
TAATAATGTA ATACCCGAAG GTATGAACTT GAGTTTCCTC ATTGCCCTTA TTTCAAATAA TGGAAATTAC
ATTATTACAT TATGGGCTTC CATACTTGAA CTCAAAGGAG TAACCGAATT AAAGTTTATT ACCTTTAATG

640     650     660     670     680     690     700
ACATGTGTTG TTACATATCC AGAAAATGGA CGTACGTTTC ATCTCACCAG GACTCTGACT GTAAAGGTAG
TGTACACAAC AATGTATAGG TCTTTTACCT GCATGCCAAG TAGAGTGGTC CTGAGACTGA CATTTCATC

710     720     730     740     750     760     770
TAGGCTCTCC AAAAAATGCA GTGCCCCCTG TGATCCATTG ACCTAATGAT CATGTGGTCT ATGAGAAAGA
ATCCGAGAGG TTTTITACGT CACGGGGGAC ACTAGGTAAG TGGATTACTA GTACACCAGA TACTCTTTCT

780     790     800     810     820     830     840
ACCAGGAGAG GAGCTACTCA TTCCCTGTAC GGTCTATTTT AGTTTTCTGA TGGATTCTCG CAATGAGGTT
TGGTCTCTCT CTCGATGAGT AAGGGACATG CCAGATAAAA TCAAAAGACT ACCTAAGAGC GTTACTCCAA

850     860     870     880     890     900     910
TGGTGGACCA TTGATGAAAA AAAACCTGAT GACATCACTA TTGATGTCAC CATTAAACGAA AGTATAAGTC
ACCACCTGGT AACTACCTTT TTTTGGACTA CTGTAGTGAT AACTACAGTG GTAATTGCTT TCATATTGAG

920     930     940     950     960     970     980
ATAGTAGAAC AGAAGATGAA ACAAGAACTC AGATTTTGAG CATCAAGAAA GTTACCTCTG AGGATCTCAA
TATCATCTTG TCTTCTACTT TGTTCITGAG TCTAAACTC GTAGTTCTTT CAATGGAGAC TCCTAGAGTT

990     1000    1010    1020    1030    1040    1050
GGCGAGCTAT GTCTGTGATG CTAGAAGTGC CAAAGGCGAA GTTGCCAAAG CAGCCAAGGT GACGCAGAAA
CGCGTTCGATA CAGACAGTAC GATCTTCACG GTTTCGGCTT CAACGGTTTC CTCGGTTCCA CTGCGTCTTT

1060    1070    1077
GTGCCAGCTC CAAGATACAC AGTGGAA
CACGGTCCGAG GTTCTATGTC TCACCTT

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/00181

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/715 C07K16/28 A61K38/17 C12N5/20
A61K39/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF CELLULAR BIOCHEMISTRY SUPPL 0, no. 18B, 1994, page 217 XP002007812 S. GREENFEDER ET AL: "Expression cloning of a cDNA encoding a novel murine Interleukin 1 receptor accessory protein" see abstract & KEYSTONE SYMPOSIUM ON TRANSMEMBRANE SIGNAL TRANSDUCTION :STRUCTURE, MECHANISMS, REGULATION OF EVOLUTION, 6 - 13 February 1994, KEYSTONE, COLORADO, USA., --- -/-	1,2, 11-15, 26,28

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Date of the actual completion of the international search

8 July 1996

Date of mailing of the international search report

12.07.96

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Le Cornec, N

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/EP 96/00181

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOLOGY PART II, vol. 150, no. 8, 15 April 1993, BALTIMORE US, page 46A XP002007498 G. D. POWERS ET AL: "Differential inhibition of murine B cell responses to iL-1 by monoclonal antibodies specific for type 1 and type 2 iL-1 receptors and a putative iL-1 receptor accessory protein" cited in the application	14,15, 21-23
X	& JOINT MEETING OF THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS AND THE CLINICAL IMMUNOLOGY SOCIETY, 21 - 25 May 1993, DENVER, COLORADO., see abstract 250	27,28
P,X	--- JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 23, 9 June 1995, MD US, pages 13757-13765, XP002007499 S.A. GREENFEDER ET AL: "Molecular cloning and characterization of a second subunit of the Interleukin 1 receptor complex" see the whole document -----	1-3,5, 11-15, 26-28

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